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1. LOW-DOSE TBI DOSE ESCALATION TO DECREASE RISKS OF PROGRESSION AND GRAFT REJECTION AFTER HEMATOPOIETIC CELL TRANSPLANTATION WITH NONMYELOABLATIVE CONDITIONING AS TREATMENT FOR UNTREATED MYELODYSPLASTIC SYNDROME OR MYELOPROLIFERATIVE DISORDERS – A MULTI-CENTER TRIAL

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2. Introduction

Hematopoietic cell transplantation (HCT) is the only curative option for patients with myelodysplastic syndromes (MDS) or myeloproliferative disorders (MPD)[1]. However, conventional HCT after intensive cytotoxic conditioning regimen has been restricted to relatively young patients, without comorbidities. We have developed a nonmyeloablative regimen that allows allogeneic HCT with HLA-matched related (MRD) or unrelated donors (URD) in older patients (up to 75 yrs old), and those with comorbidities. Results of phase I/II studies with this regimen have been very encouraging in most hematological malignancies[2-5], but results in patients with chronic myelomonocytic leukemia (CMML) and in patients with previously untreated MDS or MPD have been disappointing.

Sixty-one patients with CMML or previously untreated MDS/MPD were given grafts from MRD (n=32) or URD (n=29) after 200 cGy TBI with (n=59) or without (n=2) added fludarabine (90 mg/m²). Diagnosis were CMML (n=11), refractory anemia (RA, n=20), RA with ringed sideroblasts (RARS, n=2), RA with excess blasts (RAEB, n=11), RAEB in transformation (RAEB-T, n=4), and MPD (n=13). Forty-six patients had idiopathic diseases, while 15 patients had secondary MDS. Twenty patients achieved complete remissions 19 to 346 (median 32) days after HCT. Conversely, twenty-six of 61 patients (43%) had "***HCT failure"*** due to graft rejection (n=11) and/or disease progression (n=24) before day 200 after HCT. Specifically, 15 of 26 (58%) patients with CMML or RAEB (T) and 11 of 35 (31%) patients with RA (RS)/MPD had HCT failure before day 200. The 200-day, 1-yr and 2-yr probabilities of progression free survival (PFS) were 27%, 19% and 11%, respectively, in patients with CMML or RAEB (T), and 46%, 40%, and 37% respectively, in patients with RA (RS)/MPD.

We have recently shown that achievement of full donor T-cell chimerism was associated with a reduced risk of relapse (HR 0.5, *P*=0.002) in patients with hematological malignancies given nonmyeloablative conditioning[6]. Furthermore, a recent analysis comparing outcomes after nonmyeloablative versus myeloablative conditioning in previously untreated MDS patients has suggested that relapse/progression incidence might occur later (P=0.22) in patients given myeloablative conditioning[7]. Taken together, these data suggest that increasing the intensity of the conditioning regimen in order to prevent disease progression before establishment of full donor chimerism and occurrence of graft-versus-tumor (GVT) effects might improve outcomes of patients with CMML or untreated MDS/MPD given nonmyeloablative conditioning.

The aim of the current protocol is to decrease the risks of ***HCT failure (defined as graft rejection and/or relapse)*** in patients with CMML or with previously untreated MDS/MPD given nonmyeloablative conditioning. To do so, the protocol will escalate the TBI dose (300 cGy TBI in level 1, 400 cGy in level 2, and 450 cGy in level 3) in order to define a low-dose conditioning regimen that is associated with a day-200 incidence of ***HCT failure*** < 20%. Dose escalation will be carried out independently in two groups of patients:

Arm A – patients with MPD or MDS-RA/RARS

Arm B – patients with MDS-RAEB or CMML.

3. Background

A. Nonmyeloablative HCT with Fludarabine, Low-dose TBI and MMF/CSP

The observations that GVT effects might be able to eradicate malignant cells more effectively than chemoradiotherapy, and that in an HLA-identical transplant setting, the host-versus-graft and the graft-versus-host reactions are mediated by T-cells led to the development of a nonmyeloablative HCT approach. In preclinical canine studies, it could be shown that a nonmyeloablative conditioning regimen consisting of 200 cGy TBI, followed by post-transplant immunosuppression with mycophenolate mofetil (MMF) for 28 days and cyclosporine (CSP) for 35 days resulted in stable engraftment in DLA-identical littermates[8]. The initial transplant regimen was the same as that developed in dogs and consisted of 200 cGy TBI given on day 0, and postgrafting immunosuppression with MMF (given for 28 days) and CSP (discontinued on day 56) [2]. The stem cell sources were peripheral blood stem cells (PBSC), and donors were HLA-identical siblings. The transplant regimen was remarkably well tolerated, with the majority of eligible patients receiving their transplants in the outpatient setting. Nine of the 44 first patients (20%) given this regimen had nonfatal graft rejections [2]. In order to reduce the risk of graft rejection, fludarabine 30 mg/m^2/day × 3 days was added to the 200 cGy TBI, and the rejection rate decreased to 3% [9]. In an attempt to reduce the incidence of acute GVHD, the duration of CSP administration was extended from 56 to 77 or 180 days, while the

duration of MMF (days 0 to 27) was kept constant. This strategy was associated with a significantly reduced incidence of grade III-IV acute GVHD[10].

The same regimen of fludarabine and 200 cGy TBI was used to condition patients with 10/10-HLA-antigen matched URD [3,4]. The postgrafting immunosuppression with MMF was extended to 40 days with taper to day 96 and CSP was given for 100 days with taper through day 180. Durable engraftment was observed in 82% of PBSC (n=71) and 56% of marrow recipients (n=18). Based on this observation, all subsequent URD were given PBSC grafts. Among unrelated PBSC recipients, graft rejections were more frequently observed in patients given PBSC containing less than 6.8 x10^6 $CD34^+$ cells/kg[11]. Further, sub-optimal postgrafting immunosuppression with MMF was suggested by pharmacokinetic studies showing that the $t^{1/2}$ of mycophenolic acid, the active metabolite of MMF, was 3 hours, and its binding to IMPDH II rapidly reversible. Indeed, increasing administration of MMF from 15 mg/kg bid to 15 mg/kg tid increased the rate of durable engraftment from 82% to 95% (98/103 patients) (P=0.004)[12].

More than 800 patients ineligible for conventional HCT have been treated with this approach so far in various centers in the United States and Europe. Preliminary data in the first 451 patients receiving HCT after nonmyeloablative conditioning for hematological malignancies have demonstrated that the nonmyeloablative regimen used was safe and was associated with minimal toxicity and relatively low nonrelapse mortality, even in patients otherwise excluded from allogeneic HCT (Table 1) [5]. Given the age and status of disease of patients treated on the nonmyeloablative protocols, the rate of GHVD was not higher than in conventional HCT[13], while transplant-related toxicities and 1-yr nonrelapse mortality were significantly lower[14,15].

Table 1. Results of allogeneic HCT after a nonmyeloablative regimen consisting of 200 cGy TBI with or without fludarabine (30mg/m^2/day x 3 days) in the first 451 patients transplanted for hematologic malignancies.[5]

Patients Studied (#)	*% of Patients Acute GVHD*			*% of Patients Chronic GVHD*	*% of Mortality (2 yr Kaplan-Meier Estimates)*					*% of Survival (2 yr Kaplan-Meier Estimates)*	
					Relapse/ Progression	**NRM**					
	II	*III*	*IV*			*Overall*	*GVHD ± Infection*	*Infection*	*Other*	*Overall*	*Progr-ession-Free*
Total (n=451)	34	10	4	44	26	22	11.2	6.7	4.1	51	37
MRD (n=303)	33	10	5	43	23	22	12.5	6.5	3.0	54	40
URD (n=148)	42	9	3	45	32	22	8.5	7.1	6.4	45	31

MRD = HLA-matched related donor, URD = HLA-matched unrelated donor, NRM = nonrelapse mortality, GVHD = graft-versus-host disease.

B. Results with reduced-intensity conditioning in patients with MDS/MPD

Ho *et al.* reported results in 62 MDS patients (median age 56 yrs) given allografts from MRD (n=24) or URD (n=38) after reduced-intensity conditioning with fludarabine (150 mg/m²), oral busulfan (8 mg/kg), and alemtuzumab (100 mg total dose)[16]. Postgrafting immunosuppression consisted of CSP alone. Sixteen patients had RA, 19 RAEB, 23 RAEBT or tAML, and 4 CMML. The 1-yr probabilities of nonrelapse mortality, overall survival (OS) and PFS were 5%, 73% and 61%, respectively for MRD recipients, and 21%, 71% and 59%, respectively, for URD recipients. Twenty-six patients required DLI, given 126-1323 days after HCT, for cytogenetic (n=4) or morphologic relapse (n=6), or for decreased donor marrow chimerism (n=16). Four of 4 patients given DLI for cytogenetic relapse but none of 6 patients given DLI for morphologic relapse responded, and 14 of 16 patients given DLI for decreasing marrow chimerism achieved full donor marrow chimerism after DLI. The 2-yr cumulative incidences (including patients given DLI) of grade III-IV acute GVHD were 17% and 23% for MRD and URD recipients, respectively.

Bornhauser *et al.* reported data from 42 patients given allogeneic PBSC after conditioning with fludarabine (120 mg/m²) and busulfan (16 mg/kg, with dose adjustments to plasma levels of 900 +/- 100 ng/mL)[17]. Diagnosis

included chronic myeloid leukemia (n=4), MDS-RA (n=6), MDS-RAEB (n=11), MDS-RAEBT (n=4), untreated (n = 2) or treated AML developing from MDS (n=10; 5 in CR; 5 resistant), and CMML (n=5). GVHD prophylaxis consisted of MTX and CSP. All patients had sustained engraftment. Grade II-IV acute GVHD occurred in 54% of patients, and extensive chronic GVHD was seen in 23% of patients. The 18-month incidences of nonrelapse mortality, relapse, OS and PFS were 24%, 41%, 42%, and 35%, respectively.

Kroger *et al.* reported results from 37 MDS patients (median age 55 yrs) given grafts from MRD (n=19) or URD (n=18) after conditioning with fludarabine (120-180 mg/m²) and busulfan (8 mg/kg p.o. or 6.4 mg/kg i.v.) with (n=25) or without (n=12) added ATG[18]. GVHD prophylaxis combined CSP with MTX or MMF. Diagnoses at transplantation were RA (n=8), RAEB (n=6), RAEBT (n=13), CMML (n=3), and secondary AML (n=7). Grade II-IV acute GVHD was seen in 37% of patients, and chronic GVHD in 48%. Nonrelapse mortality was 12% in patients given graft from MRD versus 45% in patients given grafts from URD. The 3-yr probabilities of OS and PFS were 39% and 38%, respectively.

De Lima *et al.* compared HCT outcomes of 94 patients given allogeneic HCT after nonmyeloablative (fludarabine (120 mg/m²), cytarabine (4 g/m²), and idarubicin (36 mg/m²)) or reduced-intensity (fludarabine (100-150 mg/m²) and melphalan (140 or 180 mg/m²)) conditioning[19]. The 3-yr probabilities of OS were 30% in the nonmyeloablative group and 35% (NS) in the fludarabine/melphalan group. Nonmyeloablative patients had less treatment related complications, a lower incidence of grade III-IV acute GVHD (11% vs. 19%, NS), a lower nonrelapse mortality (16% vs. 39%, p=0.036), and a higher risk of relapse (53% vs. 26%, p=0.029) than patients given fludarabine plus melphalan. However, these differences could not simply be explained by differences in the intensity of the conditioning, since nonmyeloablative recipients were mainly given bone marrow from MRD while fludarabine/melphalan patients mainly received PBSC from URD, and this could have impacted both the GVHD incidence and GVT effects. In addition, 19% of patients in the nonmyeloablative group experienced graft rejection, and most of the remainder had mixed chimerism, and this might explain the reduced GVT effects.

C. Effects of TBI dose on outcome in patients given myeloablative conditioning

Two randomized and one large nonrandomized trial have explored the importance of TBI dose on HCT outcomes[20]. In total, 71 patients with AML in first remission were treated with cyclophosphamide (120 mg/kg) and either 12 Gy (*n*=34) or 15.75 Gy (*n*=37) of TBI followed by HLA-matched sibling transplantation[21]. GVHD prophylaxis consisted of CSP and MTX. The relapse rate in the group receiving the lower TBI dose was 35% compared to 12% with the higher TBI dose (*P*=0.06).

In a similar prospective randomized trial in patients with chronic myeloid leukemia in chronic phase, the relapse rate in the 57 patients treated with the lower TBI dose was 25% compared to 0% in the 59 recipients of the higher TBI dose (*P*=0.008)[22]. In both studies, nonrelapse mortality was higher with the higher TBI dose, thus balancing the reduction in relapse rates, with a result that in neither study OS was improved. Yet, these two prospective randomized studies argue that a relatively modest 30% increase in TBI dose could significantly reduce the risk of relapse, suggesting that increasing the TBI dose from 2 Gy to 3-4.5 Gy might by itself reduce the risk of relapse.

D. Retrospective comparison on outcomes of MDS patients given allogeneic HCT after myeloablative or nonmyeloablative conditioning at FHCRC

Scott *et al.* recently compared efficacy of HCT after myeloablative conditioning with busulfan [targeted (800-900 ng/mL; starting dose 1 mg/kg every 6 hours for 16 doses)] and cyclophosphamide (120 mg/kg) (n=132), or nonmyeloablative conditioning with 200 cGy TBI and fludarabine (90 mg/m², n=40) conditioning in MDS patients over 40 yrs of age[7]. The WHO distribution (highest at any time before HCT) was RA/RARS in 38% of the myeloablative and 24% of the nonmyeloablative recipients, RAEB in 31% of the myeloablative and 24% of the

nonmyeloablative recipients, and transformed AML 31% of the myeloablative and 53% of the nonmyeloablative recipients. The 3-yr probabilities of PFS were 44% in myeloablative recipients, and 28% in nonmyeloablative recipients. In multivariate analyses, there were no significant differences in OS (HR 0.9, p=0.84), PFS (HR 1.0, p=0.93), and relapse risk (HR 0.8, p=0.43) between the myeloablative versus nonmyeloablative recipients, suggesting that GVT effects were more important than conditioning intensity in preventing relapse in patients with MDS. When considering only patients who had durable complete responses to pretransplant chemotherapy, progression-free survival and progression rates did not differ between myeloablative and nonmyeloablative cohorts. This finding suggested that the intensity of transplant conditioning was not the decisive factor in preventing post-transplant progression among patients with tAML and RAEB who had responded to treatment and had less than <5% marrow myeloblasts at the time of HCT. Presumably, the pretransplant induction chemotherapy substituted for a more intensive conditioning regimen by reducing the disease burden before HCT, and the use of myeloablative conditioning offered no additional gain in progression prevention but added regimen-related toxicity.

In contrast to patients with tAML and RAEB, looking at the 51 patients with RA (not given chemotherapy before HCT), 7 of 42 (17%) patients given myeloablative conditioning versus 3 of 9 (33%) patients given nonmyeloablative conditioning progressed/relapsed. As shown in figure 1, there was a suggestion that progressions occurred later in patients given myeloablative conditioning. This observation suggests that pretransplant chemotherapy may not only reduce the disease burden, as measured by pre-HCT marrow morphology in patients with advanced MDS, but may also have effects not measurable by morphology that facilitate donor T-cell engraftment (leading to faster achievement of full donor T-cell chimerism) in the nonmyeloablative cohort and decrease risk of disease progression.

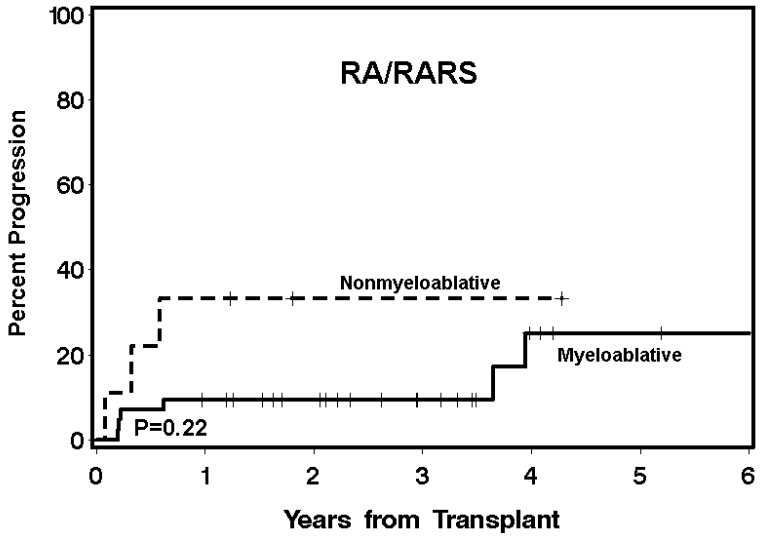

Figure 1. Cumulative incidence of progression in patients with RA/RARS given nonmyeloablative or myeloablative conditioning.

E. Results of nonmyeloablative HCT in patients with CMML or previously untreated MDS/MPD (FHCRC consortium)

Patients
Sixty-one patients with CMML or previously untreated MDS/MPD were given grafts from MRD (n=32) or URD (n=29) after 200 cGy TBI with (n=59) or without (n=2) added fludarabine. Median patient age was 59 (range, 5-73) yrs. Diagnoses were CMML (n=11), RA (n=20), RARS (n=2), RAEB (n=11), RAEB-T (n=4), and MPD (n=13). Forty-six patients had idiopathic diseases, while 15 patients had secondary MDS.

Engraftment and graft rejection

Median donor T-cell chimerism levels on days 28, 56 and 84 were 65%, 62% and 68%, respectively (Figure 2). The figures were 98%, 99% and 98% among granulocytes, and 92%, 90% and 92% among bone marrow cells. Twenty-eight patients (46%) achieved full donor T-cell chimerism 26 to 789 days (median 144 days) after HCT. Eleven patients [6 patients given grafts from MRD (including 1 of 2 patients given 200 cGy TBI only as conditioning), and five additional patients given grafts from HLA-matched URD (including 1 of 2 marrow recipients)] had graft rejection 14 to 184 (median 64 days) after HCT.

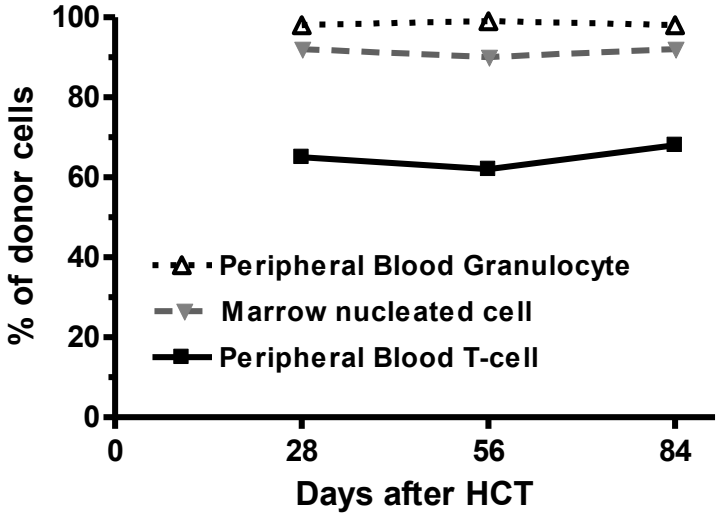

Figure 2. Engraftment kinetics.

GVHD and nonrelapse mortality

Acute GVHD of grades I, II, III and IV were seen in 2 (4%), 19 (31%), 7 (11%) and 1 (2%) patients, respectively, while extensive chronic GVHD occurred in 24 patients (40%). Nonrelapse mortality was observed in 14 patients (23%) < day 200, and in 19 patients (31%) overall (Figure 3).

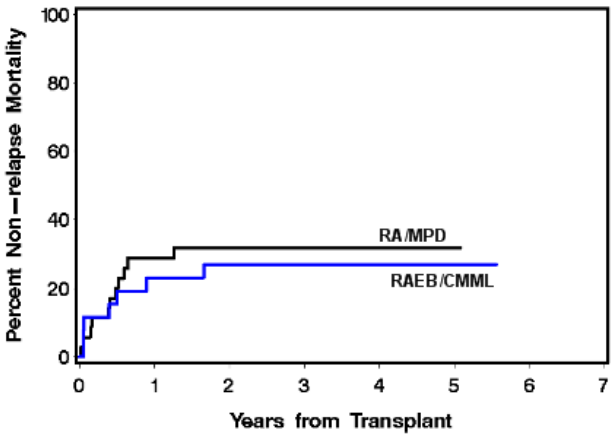

Figure 3. Nonrelapse mortality

HCT outcomes in patients with RA (RS)/MPD (n=35)

Sixteen of 35 patients (46%) achieved complete remissions 19 to 346 (median 56) days after HCT. Ten patients (29%) relapsed or progressed 117 (28-184) days after HCT, while 6 (17%) rejected their grafts 51 (23-184) days after HCT. All but one patient with graft rejection progressed. Eleven patients (31%) had "HCT failure" before day 200 (Figure 4). The 200-day, 1-yr and 2-yr probabilities of PFS were 46%, 40%, and 37% respectively (Figure 5).

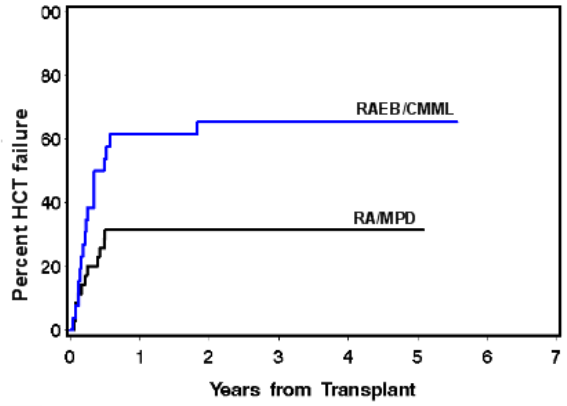

Figure 4. HCT failure (relapse or progression)

HCT outcomes in patients with CMML/RAEB (t) (n=26)

Four of 26 patients (15%) achieved complete remissions 25 to 33 (median 28) days after HCT. Seventeen patients (65%) relapsed or progressed 108 (28-1585) days after HCT, while 5 (19%) rejected their grafts 80 (14-127) days after HCT. All patients with graft rejection eventually progressed, although one CMML patient remained in complete remission for 4 yrs after graft rejection, before eventually progressing. Fifteen patients (58%) had "HCT failure" before day 200 (Figure 4). The 200-day, 1-yr and 2-yr probabilities of PFS were 27%, 19%, and 11% respectively (Figure 5).

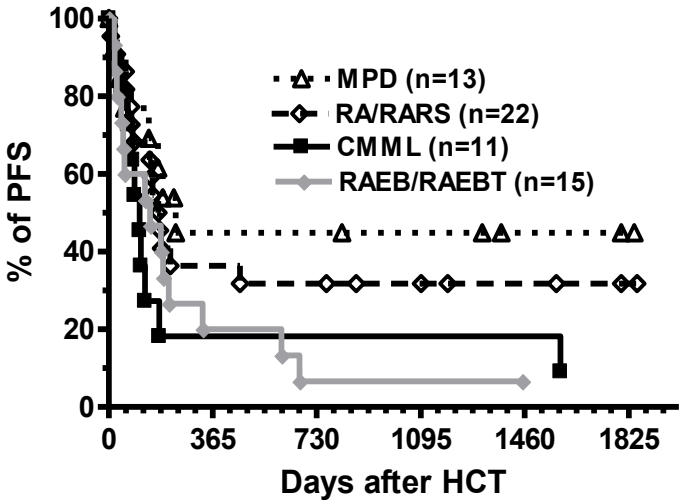

Figure 5. PFS according to diagnosis

F. Impact of achievement of full donor T-cell chimerism on relapse after nonmyeloablative conditioning
We have analyzed GVT effects in 322 patients given nonmyeloablative conditioning for hematologic malignancies[6]. Multivariate time-dependent cox regressions models were used to assess the impact of achievement of full donor T-cell chimerism on HCT outcomes. Achievement of full donor T-cell chimerism was associated with a reduced risk of relapse/progression (HR 0.5, P=0.002), and a trend for a better PFS (P=0.11).

G. Dose-response relationship of TBI with engraftment rates in a pre-clinical dog model.
A close relationship between TBI dose and rate of sustained engraftment of dog leukocyte antigen (DLA) identical marrow has been demonstrated in a preclinical canine model (Table 2). A TBI dose of 920 cGy was sufficiently immunosuppressive to permit engraftment of DLA-identical littermate marrow in 95% of dogs, even without postgrafting immunosuppression[23]. When the TBI dose was decreased to 450 cGy, 48% of dogs achieved sustained engraftment[24]. Since both host-versus-graft (rejection) and graft-versus-host reactions are mediated by T-cells after DLA-identical HCT, it was hypothesized that optimizing post-transplant immunosuppression might not only prevent GVHD, but also increase the engraftment rate. Indeed, 7 of 7 dogs given 450 cGy TBI and postgrafting with CSP achieved sustained engraftment[24]. When the TBI dose was further decreased to 200 cGy, postgrafting immunosuppression either with CSP alone or with a combination of CSP and MTX resulted in graft rejection with autologous recovery in 4 of 4 dog and 3 of 5 dogs studied, respectively[25]. Conversely, stable mixed chimerism was achieved in 11 of 12 dogs given postgrafting immunosuppression with MMF and CSP[25]. When the TBI dose was further decreased to 100 cGy, all dogs experienced graft rejection, demonstrating a delicate balance between host-versus-graft and graft-versus-host reactions[25,26]. These observations strongly support the hypothesis that increasing the dose of TBI will promote engraftment in MDS/MPD/CMML patients.

Table 2. Effect of TBI Dose and Postgrafting Immunosuppression on Engraftment of DLA-identical Marrow Grafts

Reference #	Conditioning [TBI dose (cGy)] / other	Stem cell source	Postgrafting immunosuppression / Reference	# of dogs with stable engraftment (%)*/# of dogs transplanted
		DLA-identical grafts		
[23]	920	Marrow	None	20/21 (95%)
[9]	800	Marrow	None	4/5 (80%)
[9]	700	Marrow	None	3/5 (60%)
[9]	600	Marrow	None	12/23 (52)
[9]	450	Marrow	None	10/21 (48%)
[24]	450	Marrow	CSP†	7/7 (100%)
[25]	200	Marrow	CSP†	0/4 (0%)
[25]	200	Marrow	MTX‡ + CSP†	2/5 (40%)
[25]	200	Marrow	MMF§ + CSP†	11/12 (92%)
[25]	100	Marrow	MMF§ + CSP†	0/6(0%)

*Mixed or full chimerism.
† Cyclosporine, 15 mg/kg BID PO, days -1 to 35.
‡ Methotrexate, 0.4 mg/kg IV on days 1, 3, 6 and 11.
§ Mycophenolate mofetil, 10 mg/kg BID SC, days 0 to 27.
TBI, total body irradiation.

H. What is the upper limit for nonmyeloablative TBI?

At the dose of 450 cGy, of 21 dogs studied, 14 (67%) receiving DLA-identical marrow but no growth factors survived, 10 with successful allografts (including 5 mixed chimeras) and 4 with autologous recovery; whereas 7 animals died, 5 (24%) from infections during marrow aplasia, and 2 from acute GVHD[27]. In contrast, 30 of 34 dogs (88%) given hematopoietic growth factors (G-CSF, SCF, or the combination of G-CSF and SCF) in addition to the DLA-identical marrow graft survived, 17 with successful allografts (including 10 mixed chimeras), and 13 with autologous recovery; whereas 4 (12%) died, all with infection related to marrow aplasia after rejection of the allograft[27]. Thus, 13 of 17 (76%) dogs without evidence of donor engraftment had autologous recovery. Survival was similar for recipients of G-CSF, SCF, or the combination of G-CSF and SCF. Logistic regression analyses showed a trend for improved survival in dogs given growth factors (P=0.09), no change in allogeneic engraftment (P=0.74), and an increase in autologous recovery (P=0.22). The experiments suggested that, while a TBI dose of 450 cGy was myeloablative and supralethal in dogs not given hematopoietic growth factors, autologous reconstitution occurred in 76% of dogs without donor engraftment in the presence of hematopoietic growth factors. Therefore, the upper limit dose of TBI will be 450 cGy in this protocol.

I. Current experience with 300 cGy TBI as 2nd HCT regimen in patients with graft rejection.

Three hundred cGy TBI in combination with 90mg/m^2 of fludarabine have been used as a 2nd transplant regimen in 12 patients with graft rejection both after nonmyeloablative and conventional conditioning regimens at FHCRC, Stanford University and University of Leipzig. Eleven patients had successful 2nd grafts, and one patient failed to engraft. Eight patients were alive 133 days to 4 yrs after 2nd HCT, while 4 patients died. There were no undue acute toxicities, and none of the four died as a direct consequence of the conditioning regimen. Two of the 4 patients died of pulmonary problems: one at day +456 from ARDS/pneumonitis and the other at day +36 from pre-existing advanced fungal pneumonitis. One of the other two patients died from GVHD/infections and the other from disease progression.

J. Experience with unrelated HCT after 550 cGy TBI and Cyclophosphamide.

Girgis *et al.* reported data from 110 patients with hematologic malignancies given unrelated marrow after 550 cGy TBI (given at 30 cGy/min) and cyclophosphamide (120 mg/kg)[28]. Postgrafting immunosuppression consisted of CSP, MTX and prednisone. Median patient age was 44 (range, 19 to 62) yrs. Twenty-six patients had good risk diagnosis (AML in CR1 or CML-CP), while 84 patients had poor risk diagnosis. Primary and secondary graft failure occurred in 6 patients (3 had CML, 2 had MDS, 1 had NHL) and 1 patient (with CML), respectively. Most patients with graft failure had autologous reconstitution (Adkins *et al*., personal communication), although 2 of them died because of graft failure. Fatal organ toxicity occurred in 2 patients (1 cardiac toxicity and 1 renal failure with TTP), whereas life-threatening grade IV organ toxicity occurred in 5 patients (1 mucositis, 3 SOS, 1 hemorrhagic cystitis). Incidences of grade II-IV and III-IV acute GVHD were 33% and 18%, respectively. Limited and extensive chronic GVHD were seen in 11% and 59% of patients, respectively. Cumulative rates of nonrelapse mortality in patients with good and poor risk diseases were 19% and 42%, respectively. The 3-yr probability of OS and PFS were 47% and 40% for good risk patients, and 25% and 21% for poor risk patients, respectively. We, therefore, believe it is safe to increase the dose of TBI from 200 to 300-450 cGy for patients with MDS/MPD/CMML.

K. Impact of TBI dose on testicular function.

Most of our understanding of the effects of radiation on human testis came from 2 studies [29,30] of single exposure delivered directly to the testis of normal men. Men given < 10 rad did not have testicular damage. A transient reduction in sperm concentration was observed in patients whose gonadal dose was only 15 rad, while azoospermia occurred in all patients exposed to 100 to 600 rad[29,30]. Regarding recovery of testicular function following radiation exposure, Rowley et al. reported a return of sperm counts to pre-irradiation levels after 30 months when irradiation levels were between 200 and 300 rads, and after 5 yrs or more when irradiation doses were between 400 to 600 rad[29]. Paulsen *et al.* reported similar findings although earlier recovery at approximately 30 months was observed in 2 patients given 400 rad who had sufficient long-term information[30].

L. Assessment of Pretransplant Comorbidities

Charlson Comorbidity Index (CCI) is a well-known simple index to score comorbidities which was developed to provide prediction of risks of survival after treatment of chronic medical illnesses[31]. The Seattle team used this index in 2004 to score pretransplant comorbidities among patients diagnosed with hematological malignancies and offered HCT. The CCI was helpful in predicting risks of non-relapse mortality and survival[32,33]. However, the CCI showed a limited ability in capturing comorbidities among the transplanted population. Therefore, the same authors investigated the possibility of modifying the original CCI to better capture comorbidities among transplanted patients[34]. They tried to a) better define previously identified comorbidities utilizing pretransplant laboratory data, b) investigate additional HCT-related comorbidities, and c) establish comorbidity scores that were suited for HCT. This resulted in developing a new HCT-specific comorbidity index (HCT-CI), which captured comorbidities among 62% of patients with scores >0 compared to 12% captured by the original CCI[34]. Additionally, the new index was superior to the old CCI in prediction of survival (likelihood ratio of 23.7 versus 7.1 and *c* statistics of 0.661 versus 0.561, *P*=<0.0001, respectively).

M. Impact of PBSC composition on outcomes after nonmyeloablative conditioning

Three recent studies have analyzed the impact of cell dose on outcome after nonmyeloablative conditioning. We reported data from 125 patients given ***PBSC from HLA-identical siblings*** after 2 Gy TBI with or without fludarabine. Higher number of $CD34^+$ cells transplanted was associated with better OS ($P = 0.03$)[39]. No correlations between the doses of CD3, CD4 and $CD8^+$ T cell transplanted and outcomes (and particularly acute GVHD) were identified. Cao *et al.*, using the same preparative regimen combining 2 Gy TBI with or without fludarabine, found that a higher number of transplanted $CD8^+$ T cells in the graft correlated with increased T-cell chimerism levels and better OS ($P = 0.01$) in a study analyzing combined observations in 63 patients given PBSC from either related ($n = 38$) or unrelated

(n = 25) donors[40]. We observed that higher numbers of grafted $CD34^+$ cells (the median CD34 cell dose was 6.5 x 10(6)/kg) were associated with higher levels of day 28 donor T-cell chimerism (P = 0.01), rapid achievement of complete donor T-cell chimerism (P = 0.02), and a trend for lower risk for graft rejection (P = 0.14) in 116 patients given ***unrelated PBSC*** after 2 Gy TBI and fludarabine. No correlations between doses of CD3, CD4 or $CD8^+$ T-cell transplanted and GVHD/survival were identified. Taken together, those data suggest that relatively high doses of $CD34^+$ cells should be transplanted in patients given nonmyeloablative conditioning, particularly in patients with CML, MDS or MPD[41].

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4. Proposal

The current protocol's ***primary objective*** is to decrease the incidence of day-200 HCT failure (graft rejection and /or progression) < 20% in patients with CMML or untreated MDS/MPD given related or unrelated HLA-matched HCT following nonmyeloablative conditioning. The plan is to achieve these goals by increasing the intensity of the pre-transplant TBI. The proposed conditioning regimen will continue to use fludarabine, 30 mg/m^2/day x 3 days, while the initial TBI dose will be increased from 200 cGy to 300 cGy. If this regimen fails to decrease the HCT failure rate sufficiently, the TBI dose will be increased to 400 cGy (level 2) and then to 450 cGy (level 3). Enrollment in each cohort will stop if the true rate of HCT failure on day +200 is greater than 20% (dose escalation rules; enrolment in each levels will occur in groups of 6 patients), and following patients will be included in the next cohort, or when 24 patients will be included. Dose escalation will be carried out independently in two groups of patients: Arm A – patients with MPD or MDS-RA/RARS and, Arm B – patients with MDS-RAEB or CMML. Stopping rules for the protocol will be 25% nonrelapse mortality within 200 days. ***Secondary objectives*** will be relapse/progression, PFS, kinetics of donor engraftment (chimerism), and infections.

5. Primary Objectives

By escalating the intensity of the TBI prior to HCT, the primary objective is to:

1. Decrease the incidence of day-200 HCT failure to < 20% in patients with MDS-RA (RS)/MPD and in patients with CMML/RAEB.

6. Secondary Objectives: to determine:

1. The rate of relapse/progression in patients with MPD or MDS-RA and those with CMML or MDS-RAEB.
2. The probability of PFS in patients with MPD or MDS-RA and those with CMML or MDS-RAEB.
3. The kinetics of donor engraftment.
4. The incidence of infections.

7. Patient Selection

7.1 Inclusion Criteria

(A) Patients aged ≥50 and < 75 yrs with CMML, or previously untreated MDS or MPD as described in Sections 7.1.1, 7.1.2 and 7.1.3, 7.1.4, and 7.1.5.

(B) Patients aged < 50 yrs at high risk for regimen related toxicity using standard high dose regimens. Factors considered high risk include pre-existing conditions such as a chronic disease affecting kidneys, liver, lungs, or heart or previous failed HCT.

(C) An HLA-identical related or an HLA-matched unrelated donor (FHCRC matching allowed will be Grade 1.0 to 2.1 (**Appendix O**)) is available (Refer to section 8.1 and 8.3).

(D) Recovery from the effects of previous chemotherapy, with a minimum of 21 days from initiation of last therapy. Hydroxyurea or anagrelide may be used to manage elevated cell

counts in patients up to the time they begin therapy under this protocol.

(E) Patients < 12 yrs of age must be discussed on a case by case basis with the PI of the protocol (Brenda Sandmaier M.D. (206-667-4961)) prior to protocol registration.

(F) A signed informed consent form or minor assent form.

7.1.1 MDS

(A) MDS classifiable by the WHO system (see appendix R) as RA, RARS, refractory cytopenia with multilineage dysplasia (RCMD), RCMD and ringed sideroblasts (RCMD-RS) or RAEB.

(B) No previous myelosuppressive therapy. For the purpose of this protocol myelosuppressive chemotherapy will be defined as chemotherapy given with the intent of inducing a complete remission (e.g. standard 7+3, HIDAC, or mylotarg).

(C) Patients must have < 10% marrow blasts. Fewer than 10% marrow blasts must be documented by marrow examination within 3 weeks of initiation of conditioning.

7.1.2 CMML

(A) Patients with CMML1 who have not received myelosuppressive therapy must have < 10% marrow blasts. Fewer than 10% marrow blasts must be documented by marrow examination within 3 weeks of initiation of conditioning.

OR

Patients with CMML who have progressed beyond CMML1 and have received myelosuppressive chemotherapy must have <5% marrow blasts. Fewer than 5% marrow blasts must be documented by marrow examination within 3 weeks of initiation of conditioning.

7.1.3 MPD

(A) Patients with polycythemia vera with persistent thrombotic or hemorrhagic complications despite conventional therapy, or who have progressed to postpolycythemic marrow fibrosis.

(B) Patients with essential thrombocythemia with persistent thrombotic or hemorrhagic complications despite conventional therapy, or who have progressed to myelofibrosis.

(C) Chronic idiopathic myelofibrosis with peripheral blood cytopenias.

(D) Patients must have < 10% marrow blasts. Fewer than 10% marrow blasts must be documented by marrow examination within 3 weeks of initiation of conditioning.

(E) No previous myelosuppressive therapy. For the purpose of this protocol myelosuppressive chemotherapy will be defined as chemotherapy given with the intent of inducing a complete remission (e.g. standard 7+3, HIDAC, or mylotarg).

7.1.4 Atypical CML

(A) Philadelphia chromosome-negative patients with a diagnosis of atypical CML.

(B) Patients must have < 10% marrow blasts. Fewer than 10% marrow blasts must be documented by marrow examination within 3 weeks of initiation of conditioning.

(C) No previous myelosuppressive therapy. For the purpose of this protocol myelosuppressive chemotherapy will be defined as chemotherapy given with the intent of inducing a complete remission (e.g. standard 7+3, HIDAC, or mylotarg).

7.1.5 Paroxysmal Nocturnal Hemoglobinuria (PNH)

Patients with the non-aplastic form of PNH (cellular bone marrow) who have had a history of life-threatening complications of their disease including thrombotic events, severe hemolysis or Budd Chiari syndrome are eligible. Other patients may be considered following approval at PCC and approval by the protocol Principal investigator.

7.2 Exclusion Criteria

(A) Organ dysfunction as defined by the following:

1. Symptomatic coronary artery disease or cardiac ejection fraction < 35% (or, if unable to obtain ejection fraction, shortening fraction of <26%). If shortening fraction is <26% a cardiology consult is required with the PI having final approval of eligibility. Ejection fraction is required if age > 50 years or there is a history of anthracycline exposure or history of cardiac disease.
2. DLCO <35%, TLC <35%, FEV1 <35% and/or receiving supplementary continuous oxygen. The FHCRC PI of the study must approve of enrollment of all patients with pulmonary nodules.
3. Liver function abnormalities: Patient with clinical or laboratory evidence of liver disease will be evaluated for the cause of liver disease, its clinical severity in terms of liver function, bridging fibrosis, and the degree of portal hypertension. The patient will be excluded if he/she is found to have fulminant liver failure, cirrhosis of the liver with evidence of portal hypertension, alcoholic hepatitis, esophageal varices, a history of bleeding esophageal varices, hepatic encephalopathy, uncorrectable hepatic synthetic dysfunction evinced by prolongation of the prothrombin time, ascites related to portal hypertension, bacterial or fungal liver abscess, biliary obstruction, chronic viral hepatitis with total serum bilirubin >3mg/dL, or symptomatic biliary disease.

(B) Bone marrow documenting blast count $\geq$10% or $\geq$5% in CMML patients who have progressed beyond CMML1 and received myelosuppressive chemotherapy

(C) Patients with active non-hematologic malignancies (except non- melanoma skin cancers).
This exclusion does not apply to patients with non-hematologic malignancies that do not require therapy

(D) Patients with a history of non-hematologic malignancies (except non-melanoma skin cancers) currently in a complete remission, who are less than 5 years from the time of complete remission, and have a >20% risk of disease recurrence.

(E) Presence of $\geq$5% circulating leukemic blasts (in the peripheral blood) detected by standard pathology.

(F) Active CNS involvement of disease (if LP requirement, see **Appendix N).**

(G) Karnofsky performance score < 70% or Lansky-Play Performance score < 70 for pediatric patients

(H) Life expectancy severely limited by diseases other than malignancy

(I) Fungal infections with radiological progression after receipt of amphotericin product or active triazole for > 1 month

(J) Active bacterial infection

(K) Patients of fertile age who refuse contraception for a twelve month period post-transplant

(L) Females who are pregnant or breastfeeding

(M) HIV seropositivity

(N) Severe psychological illness such as major psychosis (e.g. schizophrenia), major bipolar depression, or suicidal situational depression.

8.0 Donor Eligibility

8.1 Inclusion Criteria – MRD

(A) Related to the patient and is genotypically or phenotypically HLA-identical.

(B) Donor age < 75 yrs unless cleared by institutional P.I

(C) Capable of giving written, informed consent.

(D) Donor must consent to PBSC mobilization with G-CSF and apheresis

8.2 Exclusion Criteria – MRD

(A) Identical twin

(B) Any contra-indication to the administration of subcutaneous G-CSF at a dose of 16mg/kg/d for five consecutive days

(C) Serious medical or psychological illness

(D) Pregnant or lactating females

(E) Prior malignancy within the preceding five yrs, with the exception of non-melanoma skin cancers.

(F) HIV seropositivity

8.3 Inclusion Criteria – URD

(A) **FHCRC matching allowed will be Grades 1.0 to 2.1 (Appendix O)**: Unrelated donors who are prospectively:

i) Matched for HLA-A, B, C, DRB1 and DQB1 by high resolution typing;
ii) ***Only a single allele disparity*** will be allowed for HLA-A, B, or C as defined by high resolution typing (see **Appendix O for other donor selection details**).

(B) Patient and donor pairs homozygous at a mismatched allele in the graft rejection vector are considered a two-allele mismatch, i.e., the patient is A*0101 and the donor is A*0102, and this type of mismatch is not allowed.

(C) Only G-CSF mobilized PBMC only will be permitted as a HSC source on this protocol.

(D) Donor must consent to PBSC mobilization with G-CSF and apheresis. Bone marrow unrelated donors are not eligible for this protocol

8.4 Exclusion Criteria-URD

(A) **A positive anti-donor cytotoxic crossmatch is an absolute donor exclusion.** Donors are excluded when preexisting immunoreactivity is identified that would jeopardize donor hematopoietic cell engraftment. This determination is based on the standard practice of the individual institution. The recommended procedure for patients with 10 of 10 HLA allele level (phenotypic) match is to obtain a panel reactive antibody (PRA) screens to class I and class II antigens for all patients before HCT. If the PRA shows >10% activity, then flow cytometric or B and T cell cytotoxic cross matches should be obtained. The donor should be excluded if any of the cytotoxic cross match assays are positive. For those patients with an HLA Class I allele mismatch, flow cytometric or B and T cell cytotoxic cross matches should be obtained regardless of the PRA results.
(B) Marrow donors
(C) Donors who are HIV-positive and/or medical conditions that would result in increased risk to the donor G-CSF mobilization and G-PBMC collections.
(D) Serious medical or psychological illness
(E) Pregnant or lactating females
(F) Prior malignancy within the preceding five yrs, with the exception of non-melanoma skin cancers.
(G) HIV seropositivity

9. Informed Consent

A conference will be held with the patient and family to discuss this study and alternative treatments available for the underlying disease. A separate conference will be held for the donor. The conference will be conducted by the outpatient-attending physician. All potential risks associated with the use of fludarabine, low dose TBI, immunosuppressive drugs, HCT, GVHD, infections, rejection, disease progression/recurrence, risk of infertility and DLI should be discussed as objectively as possible. Specifically, the advantages and risks of this approach in comparison to non transplant strategies and myeloablative HCT should be discussed. Informed consent from the patient will be obtained using a form approved by the Institutional Review Board (IRB) of the Fred Hutchinson Cancer Research Center and the local IRB if the patient is treated in a collaborating institution.

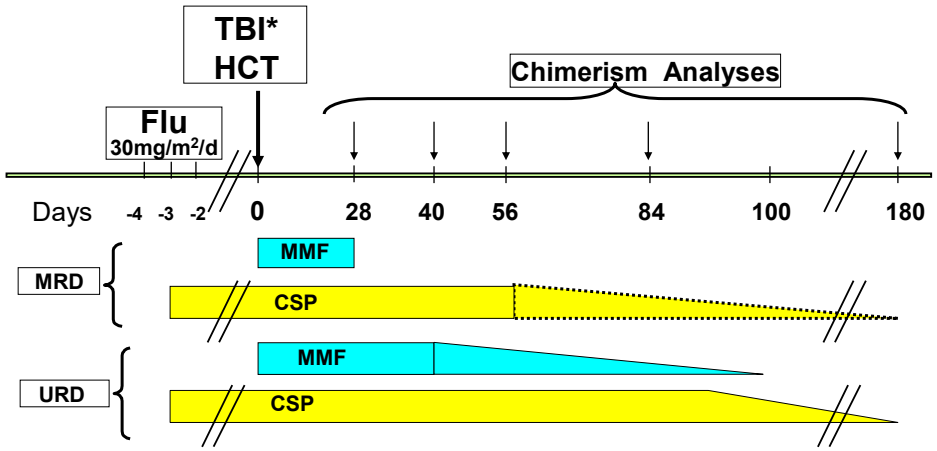
10. Protocol Registration

FHCRC patients: Eligible patients will be identified by the Clinical Coordinators Office. Patients will be registered with the Registration Office (206-667-4728) between 8:30 am and 4:00 PM, Monday through Friday. After hours, the Registration office can be reached by paging (206) 995-7437.

Collaborating institutions: Eligible patients will be identified by the principal investigator of the collaborating institution who will register the patient with the FHCRC. Registration will include completion of the eligibility checklist/demographic form (**Appendix L**). This form will be faxed to the trial coordinator (206-667-5378). Questions regarding eligibility or protocol information should be directed to Brenda Sandmaier, M.D. (206-667-4961).

11. Plan of Treatment

A. Outline of Treatment Plan (refer to **Figure 6** and **Table 3**)



FLU: 30 mg/m² x 3 days (days -4, -3, -2).
TBI: Single fraction at 7 cGy/min. 300 cGy (level 1), 400 cGy (level 2), 450 cGy (level 3).
CSP: MRD: 5.0 mg/kg po q12hrs days-3 to + 56 then taper to day +180.
URD: 5.0 mg/kg po q12hrs days-3 to + 100 then taper to day +180.
MMF: MRD: 15 mg/kg po q12hrs days 0 to + 27.
URD: 15 mg/kg po q8hrs days 0 to + 40, then taper to day + 96.

Figure 6. Protocol treatment Schema

B. Cytoreduction: Cytoreduction, radiation therapy or both may be given by the referring physician or the attending physician as determined on clinical grounds or to meet eligibility requirements of the protocol for patients with CMML. However, no intensive chemotherapy can be given within 3 weeks prior to initiating conditioning (see exclusion criteria). The need for this therapy should be discussed with the principal investigator. The referring oncologist may be asked to administer this therapy.

C. Discontinuation of Hydroxyurea and other medications: For patients who are under hydroxyurea prior to HCT, this medication should be discontinued on day -2. Thalidomide, lenalidomide, arsenic trioxide, imatinib mesylate and farnesyl transferase inhibitors should be discontinued at least 3 weeks prior to HCT.

D. Conditioning Regimen: (refer to **Figure 6** and **Tables 3 A/B**)

- Days -4, -3 and –2: Fludarabine 30mg/m^2/day IV.
- Day 0: TBI 300 cGy (level 1), 400 cGy (level 2) or 450 cGy (level 3) at 6-7 cGy/min from linear accelerator followed by HCT. Regardless of the actual time of TBI administration on DAY 0, immunosuppression should be given per schedule and prior to the infusion of PBSCs.

Table 3A. Patients with **MRD** - Conditioning Schema and Immunosuppression Schedule

Day Number	*-4*	*-3*	*-2*	*-1*	*0*	*+1*	*+ 28*	*+56*	*+180*
Fludarabine	**X**	**X**	**X**						
TBI					300 cGy (level 1) 400 cGy (level 2) 450 cGy (level 3)				
PBSC					Infusion				
CSP		START	→	→	→	→	→	TAPER	STOP
MMF					START[a]	**BID**	STOP		

[A] The first dose of MMF is to be given 4-6 hours after the stem cell infusion.

Table 3B. Patients with **URD** - Conditioning Schema and Immunosuppression Schedule

Day Number	*-4*	*-3*	*-2*	*-1*	*0*	*+1*	*+40*	*+96*	*+100*	*+180*
Fludarabine	**X**	**X**	**X**							
TBI					300 cGy (level 1) 400 cGy (level 2) 450 cGy (level 3)					
PBSC					Infusion					
CSP		START	→	→	→	→	→	→	TAPER	STOP
MMF					START[a]	**TID**	TAPER[b]	STOP		

[A] The first dose of MMF is to be given 4-6 hours after the stem cell infusion.
[B] Taper of MMF will be a ~10% dose reduction per week x 8 weeks.

E. PBSC infusion

G-CSF mobilized PBSC from MRD or URD will be the only source of hematopoietic stem cells. Two 12-liter leukaphereses on consecutive days, day –1 and day 0, will be obtained, and cells will be infused together on day 0 following TBI. Refer to institutional practice guidelines for methods of infusion. If the CD34 cell dose is $<5.0 \times 10^6$/kg after the second collection, a third day collection should be added, and extra dose of G-CSF should be given to the donor before the final collection (see also section G).

F. Immunosuppression

MRD

- Day –3: CSP at 5.0mg/kg PO q12hrs, continue to day +56 and taper to day +180. CSP should be routinely taken at 9:00 a.m. and 9:00 p.m.
- Day 0: **After** HCT on day 0, MMF will be given at 15mg/kg PO at 4-6 hours after PBSC infusion is complete, then to be given at 15mg/kg PO q12hrs until day +27 and then discontinued.

URD

- Day –3: CSP at 5.0mg/kg PO q12hrs, continue to day +100 and taper to day +180. CSP should be routinely taken at 9:00 a.m. and 9:00 p.m.

- Day 0: **After** HCT on day 0, MMF will be given at 15mg/kg PO at 4-6 hours after PBSC infusion is complete, then to be given at 15mg/kg PO q8hrs until day +40, and then taper to day +96.

a. CSP

1. Starting Dose:

A. Adult dose. CSP (Neoral is preferred) is given orally at 5.0mg/kg q12hrs PO (based on adjusted body weight) from day –3 until day +56 (MRD) or +100 (URD). Dose should be adjusted to maintain a high therapeutic CSP level as discussed below. If there is nausea and vomiting at any time during CSP treatment the drug should be given intravenously at the dose that was used to obtain a therapeutic level. The conversion from oral CSP (Neoral) to intravenous cyclosporine = oral cyclosporine dose divided by 2.5 equals IV dose. Use CSP levels to further adjust the dose. The formulation of CSP can be changed to Sandimmune if nausea and vomiting are persistent.

B. Pediatric Dose: Due to the variable and increased metabolism in children, CSP will be started intravenously at the following doses at day –3 to be adjusted to maintain a therapeutic level as specified below. CSP trough level should be obtained on day 0 and CSP dose adjusted to ensure therapeutic levels. The patient may be changed to oral CSP after HCT when he or she is able to take oral medications. Dose should be adjusted to maintain high therapeutic levels as discussed below. If nausea or vomiting occur at any time during CSP treatment, CSP should be administered intravenously at the dose that was used to obtain a therapeutic level. The conversion from oral CSP (Neoral) to intravenous cyclosporine = oral cyclosporine dose divided by 2.5 equals the IV dose. Use CSP levels to further adjust the dose.

- Age ≤6 years old: 1.6mg/kg IV q8hrs
- Age >6 years old: 2.0mg/kg IV q12hrs.

Continuous infusion of cyclosporine may be appropriate due to toxicity or variable levels. Infuse total daily dose over 22-24 hours by continuous IV infusion.

2. In the absence of GVHD, for MRD recipients: CSP is to be tapered from day +56 and discontinued on day + 180; for URD recipients: CSP is to be tapered from day +100 and discontinued on day + 180. The referring physician, who will receive explicit instructions and guidelines for detecting and managing GVHD, will manage this. Modifications of the taper schedule may be indicated if significant disease progression occurs early post-transplant (see section K below).

3. Blood pressure, renal function (serum creatinine, BUN), electrolytes and magnesium need to be followed at least three times per week during the first month, twice weekly until day +100, then once per week until CSP is stopped, unless clinical circumstances suggest the need for more frequent evaluations.

4. CSP Dose Adjustments: Initial high Cyclosporine (CSP) doses are required based on the pre clinical nonmyeloablative canine studies, which used an equivalent dose to establish an allograft. After day +28, CSP levels typical for unrelated HCT will be targeted. Dose reduction should only be made if CSP toxicity is present, and/or levels exceed values provided in Table 4. There are two methods for calculating CSP levels. Table 4 provides desired levels for specific methods. To avoid inadequate immune suppression, dose reductions should be conservative. Therapeutic levels of CSP should be maintained.

Table 4: CSP Dose Adjustment by Levels

	CSP Level to Target Using LC-MS/MS Method	**CSP Level to Target Using Immunoassay Method**
Day "0" – Day +28 **Whole blood "trough" (11-12 hrs from prior dose)**	400 ng/ml	500 ng/ml (upper end therapeutic range for this method)
After Day +28	120 – 360 ng/ml	150 – 450 ng/ml
Levels >480 ng/ml by LC-MS/MS Method • **with or without CSP toxicity** • **decrease GFR ≥50%** • **increase creatinine 2x baseline due to CSP**	25% dose reduction	N/A
Levels >600 ng/ml by Immunoassay Method • **with or without CSP toxicity** • **Decrease GFR ≥50%** • **increase creatinine 2x baseline due to CSP**	N/A	25% dose reduction
Patients on Hemodialysis	320 ng/ml	400 ng/ml

5. CSP Monitoring: Further CSP determinations should be performed on a twice weekly basis for the first month and then weekly until day +100 unless high levels are detected (i.e., >600 ng/ml), or toxicity is suspected in which case more frequent monitoring will be performed as clinically indicated. Routine monitoring of CSP will not be required for patients on a CSP taper unless clinically indicated.

6. Drugs Interactions: Drugs that may affect CSP levels are shown in **Table 5**.

Table 5: Drugs Affecting CSP Level

Decrease CSP Levels	Increase CSP Levels	Enhance Potential for Nephrotoxicity
carbamazepine	azithromycin	Aminoglycosides
nafcillin	diltiazem	Loop diuretics (furosemide)
octreotide	alcohol	Amphotericin formulations
phenobarbital	acetazolamide	
phenytoin	caspofungin	
primidone	clarithromycin	
rifampicin	colchicine/diltiazem	
sulfonamides	doxycycline	
trimethoprim	erythromycin	
metoclopramide	fluconazole*	
	fluoroquinolones	
	imipenem	
	itraconazole*	
	ketoconazole	
	nicardipine	
	nifedipine	
	verapamil	
	voriconazole	

**Discontinuation of fluconazole or itraconazole may lower CSP levels, and if used as antifungal prophylaxis changes in these drugs should be avoided during the first month post-transplant.*

b. MMF

1. Initiating MMF Therapy: Oral administration of MMF will be given based on adjusted body weight at 15mg/kg q12hrs (30mg/kg/day) for MRD recipients, and at 15mg/kg q8hrs (45mg/kg/day) for URD recipients, from **the evening of day 0** (i.e. first dose to follow 4-6 hours after HCT). Doses will be rounded to the nearest 250mg (capsules are 250mg). If there is nausea and vomiting at any time preventing the oral administration of MMF, MMF should be administered intravenously based on adjusted body weight at 15mg/kg q12hrs for MRD recipients, or 15mg/kg q8hrs for URD recipients.

2. Tapering of MMF: MRD recipients: MMF will be given daily at 15mg/kg q12hrs through day +27, and then in the absence of GVHD, discontinued on day 28.URD recipients: MMF will be given daily at 15mg/kg q8hrs through day +40 post transplant, and then in the absence of GVHD, MMF should be tapered at day +40 by 10%/week x 8 weeks and discontinued on day +96.

3. Maintaining MMF: Markedly low (<40%) donor T-cell chimerism after HCT may indicate impending graft rejection[35]. MMF should be continued at full dose or, if MMF taper has been initiated, reinstitution of full dose MMF should occur. Consideration of graft salvage with use of pentostatin + DLI (as per protocol 1825) or other institutional protocol should be considered.

4. Guidelines for MMF dose adjustment due to drug toxicity:
   - If in the clinical judgment of the investigator the observed toxicity is related to MMF administration, a dose adjustment may occur. The discontinuation of MMF at any point should be discussed with the Study PI and should be documented in the permanent medical record and all Case Report Forms (CRF).
   - Gastrointestinal Toxicity. Severe gastrointestinal toxicities such as gastrointestinal hemorrhage have been very rare after nonmyeloablative HCT. In the event of gastrointestinal toxicity that requires medical intervention including medication for control of persistent vomiting or diarrhea that is considered to be due to MMF after day +28, a 20% dose reduction will be made or the

drug may be given IV. If severe refractory diarrhea or overt gastrointestinal bleeding occurs, MMF may be temporarily stopped. The MMF should be restarted at 20% reduced dose when the underlying toxicity subsides.

- Neutropenia. Based on previous experience in patients after nonmyeloablative HCT, dose adjustments are not likely to occur because of hematopoietic adverse effects, in particular neutropenia. A thorough evaluation of neutropenia should occur including peripheral blood chimerism studies, marrow aspiration and review of marrow suppressive medications (e.g. bactrim, ganciclovir). If all other potential causes of marrow toxicity are ruled out, dose adjustments will only be made for grade IV neutropenia that persists after day +28 post-transplant. Dose reductions should be conservative (20%). After day +21, the use of G-CSF will be permitted for neutropenia. For severe toxicity related to MMF (grade IV neutropenia > 5 days refractory to G-CSF), MMF may be decreased and if neutropenia persists, MMF can be stopped. The MMF should be restarted at 20% reduced dose when the underlying toxicity subsides.

G. Collection and infusions of Donor PBSC (see also **section 11.L**)

MRD

G-CSF administration to Donors

From day -4 to day 0, all PBSC donors will receive G-CSF at a dose of 16 µg/kg/day for 5 consecutive days. G-CSF will be administered by a subcutaneous daily injection. These doses will be administered before 10:00 a.m. each day in the Outpatient Department. The schedule of G-CSF administration and PBSC collections can only be ascertained once day 0 is identified. Once a treatment regimen schedule has been fixed and the schedule of G-CSF administration and PBSC collections made, this has to be confirmed with the personnel in the apheresis room. Day 0 should be fixed on a Tuesday-Thursday.

PBSC collection

Donors will preferably undergo vein-to-vein collections or may receive an appropriate catheter inserted on or before day of apheresis. PBSCs will be collected in the afternoon of day -1, stored in the refrigerator at 4ºC overnight. A second collection will be performed the following afternoon and both collections will be transfused on day 0. If $< 5 \times 10^6$ CD34+ cells/kg are collected an additional day of collection will be performed. If PBSCs cannot be collected by a vein-to-vein technique, a percutaneous Mahurkar catheter will be inserted. General procedures will include the use of a standard apheresis machine (COBE Spectra, Lakewood Colo.), and processing up to 16 liters of whole blood during the collection. The plan for PBSC collection is shown in **Table 6**.

Immunophenotyping of the G-PBMC product will be performed by the cryobiology laboratory and will include T-cells and their subsets, monocytes, and NK cells.

Table 6. Treatment Schema for Related Donors

Day	**-4**	**-3**	**-2**	**-1**	**0**
G-CSF 16 µg/kg/SQ	X	X	X	X	X
PBSC collection				X	X

URD

G-CSF Administration to Donors
Timing of PBSC collection is prearranged through the NMDP. Day 0 should be fixed on a Monday-Thursday when possible. G-CSF will be administered by subcutaneous injection to the unrelated donor starting 5 days prior to the day of HCT (see **Table 7**) as per NMDP protocol. Donors will receive approximately 10μg/kg of G-CSF each day of mobilization. A 12-liter apheresis will be obtained on day –1 and possibly on day 0 for a total of 12 to 24 liters of apheresis collection that will be infused on day 0.

Table 7. Treatment Schema for Unrelated Donor

Day	*-5*	*-4*	*-3*	*-2*	*-1*	*0*
G-CSF (~10 μg/kg)	X	X	X	X	X	
PBSC collection					X	X

PBSC Collection
HCT scheduling and collection is arranged through the NMDP. The schedule of G-CSF administration and collection of PBSC is determined as per NMDP protocol. The physician responsible for hematopoietic cell collection will obtain informed consent from the donor.

Immunophenotyping of the G-PBMC product will be performed by the cryobiology laboratory and will include T-cells and their subsets, monocytes, and NK cells.

Collection of DLI. Donor lymphocytes will be collected from unrelated donor G-PBMC products prior to transplant for potential future use of DLI on other protocol or treatment plans. A portion of the PBSC product (10%) from unrelated donors will be frozen according to standard cryopreservation for DLI. Unrelated donor PBSC products will be frozen in an aliquot of 1.0×10^7 CD3+ cells/kg.

MRD/URD

PBSC infusion
All patients will receive unmodified PBSC infusion on day 0 of the treatment regimen (Refer to institutional practice guidelines for methods of infusion).

H. ABO Incompatibility

All patients with ABO incompatibility should be evaluated and treated as according to the standard practice of the individual institution. Recommendations are provided in **Appendix D**. It should be noted that two cases of recipient host red blood cell hemolysis have been documented in patients with minor ABO mismatch with their donor[36]. The suspected cause is donor anti-host hemagglutinin production from "passenger lymphocytes" in the donor PBSC that may expand post-transplant. Therefore, these patients should be monitored and treated aggressively when there is any evidence of hemolysis.

I. Post-transplant Growth Factors

Patients should in general not receive post-transplant growth factors during the first 21 days after HCT. Growth factors should not be given unless grade IV neutropenia develops or persists past day +21 post-transplant (ANC <500/L).

J. Infection Prophylaxis

Recommended prophylaxis for PCP, VZV, and HSV are listed in **Appendix E** with the modification that PCP, VZV, and antifungal prophylaxis should be continued if the patient is receiving treatment for chronic GVHD. Since antifungal prophylaxis strategies are evolving, patients may receive antifungal prophylaxis as per the standard practice of the treatment institution. Standard CMV monitoring and prophylaxis should commence at the time of transplant and should continue until discontinuation of immunosuppression. Patients who reject their graft can discontinue this infection prophylaxis.

K. Modifications of Immunosuppression for Low Donor T-cell Chimerism (impending graft rejection) and Disease Progression

This section provides guidelines for management of patients with low donor chimerism and disease progression. Those patients with significant progression of disease as defined in **Table 8** will undergo more rapid reduction of immunosuppression. DLI will not be given for progressive or relapsed disease on this protocol, and patients with relapse or progression would be eligible for other ongoing DLI protocols or treatment plans. Note that persistence of disease in itself does not mandate accelerated taper of immunosuppression.

1. **Definition of mixed donor/host chimerism, engraftment, graft failure and rejection**. For the purposes of this protocol, *mixed chimerism* will be defined as the detection of donor T-cells (CD3+) and granulocytes (CD 33+), as a proportion of the total T-cell and granulocyte population, respectively, of greater than 5% and less than 95% in the peripheral blood. *Full donor chimerism* is defined as ≥ 95% donor CD3+ T-cells. Mixed or full donor chimerism will be evidence of *donor engraftment. Increasing donor chimerism* is defined as an absolute increase of 20% of CD3+ donor T-cells over the previous chimerism evaluation. Low donor chimerism is defined as ≤ 40% CD3+ donor T-cells any time after HCT. Low donor chimerism should always be confirmed with repeat peripheral blood T-cell and NK cell chimerism analysis. A DNA-based assay that compares the profile of amplified fragment length polymorphisms (ampFLP) (or FISH studies or VNTR) of the patient and donor will be used to quantitate chimerism of sorted peripheral blood T-cells (CD3+) and granulocytes (CD 33+). The same assay should be used in a given patient for repeated studies of chimerism. This DNA-based analysis will also be performed on the whole nucleated cell fraction from marrow aspirates. Therapeutic decisions (e.g. pentostatin + DLI as per protocol 1825) will be made based on the results of sorted T-cell studies of *peripheral blood.* For the purposes of this protocol, *rejection* is defined as the inability to detect or loss of detection of greater than 5% donor T-cells (CD3+) as a proportion of the total T-cell population, respectively, after nonmyeloablative HCT. Also for the purposes of this protocol, *graft failure* is defined as grade IV thrombocytopenia and neutropenia after day +21 that lasts > 2 weeks and is refractory to growth factor support.

2. **Evaluation of chimerism.** Patients will have peripheral blood and whole bone marrow evaluations for chimerism at various time points through one year post transplant. If the patient has not obtained > 95% donor chimerism in CD+3 by one year continue to evaluate through 5 years post transplant as clinically necessary. Peripheral blood will be sorted to evaluate T-cell (CD+3), and NK cell (CD56) compartments. (See Patient Post Transplant Evaluation section for instructions and exceptions).

3. **Continuation of immunosuppression.** In the setting of low (≤40%) donor chimerism, immunosuppression should be continued or reinitiated at full dose so that DLI can be administered after pentostatin administration according to protocol 1825. If there is disease progression in the setting of low donor chimerism, the algorithm for disease progression (below) should be followed. Patients who reject their graft will not be offered DLI and may be eligible for a second allogeneic transplant on other protocols.

4. **Disease progression.** Evidence of disease progression (as defined in **Table 8**) will be an indication for therapeutic intervention. In part, this will be dependent on where a patient is relative to the standard tapering schedule. If the attending physician believes that the patient requires very aggressive therapy, the case will be presented to the institutions' patient review committee (such as patient conference care at the FHCRC). Otherwise, priority should be given to rapid reduction of immunosuppression, option (a) below. Therapeutic options include:

Table 8. Definition of Disease Progression.

Disease	*Progression*
MDS/ CMML	• Any evidence by morphologic or flow cytometric evaluation of the bone marrow aspirate of **an incremental increase in 5% blasts**
Agnogenic Myeloid Metaplasia / Atypical CML	• Any evidence of blastic transformation.
Polycythemia Vera and Essential Thrombocythemia	• Progressive erythrocytosis, thrombocytosis, or evidence of leukemic transformation.

a. Early discontinuation of immunosuppression (prior to day +100). This should be considered the first therapeutic maneuver. If there is no GVHD, MMF is to be stopped, and CSP tapered over 2 weeks. Bone marrow aspirate and blood chimerism studies will be performed when off immunosuppression after 2 weeks. If there is no response to stopping immunosuppression and there is no GVHD, patients will be considered disease treatment failure. DLI as treatment for disease progression or relapse will not be offered on this protocol, however patients may treated according to other research protocols such as FHCRC protocol 1803. If there is progressive disease that requires therapy before 4 weeks, or progressive disease occurs despite onset of GVHD, then patients can be treated off protocol with DLI or be considered for (c) or (d) below.

b. Early discontinuation of immunosuppression (between day +100 and +180). If there is no GVHD, CSP is to be stopped. Bone marrow aspirate and blood chimerism studies will be performed when off immunosuppression after 2 weeks. If there is no response to stopping immunosuppression, and there is no GVHD, patients will be considered as treatment failure. DLI will not be offered for disease progression or relapse on this protocol but patients can be treated off protocol with DLI or be considered for (c) or (d) below.

c. Treatment with chemotherapy. Conventional chemotherapy should be considered in the setting of life threatening disease progression. Patients in this situation would be considered treatment failures. After therapy is completed chimerism should be evaluated and the administration of DLI off protocol considered.

d. Conventional allogeneic HCT. This option should be discussed with the institutions' patient review committee and the principal investigator. Patients who undergo conventional allogeneic HCT will be removed from this protocol at that time.

L. Immunophenotyping of PBSC Administration Guidelines

Immunophenotyping of the PBSC product will be performed by the cryobiology laboratory and will include T-cells and their subsets, monocytes, and NK cells. Unrelated donor PBSC products will be frozen in an aliquot of 1.0 x 10^7 CD3+ cells/kg (for use as DLI if needed). Infusions of cryopreserved PBSC should be performed as per standard practice for outpatient PBSC.

12. Assessment of Disease Responses (see also Appendix H)

12.1 General guidelines

(A) Any assessment of response must include a bone marrow aspirate. Patients with myelofibrosis will also require a biopsy.
(B) All bone marrow aspirates must also have cytogenetics analysis. For some patients, there may be additional disease-specific FISH or PCR-assessed molecular markers that can be used to determine response.
(C) Regimen-related toxicity, for example due to drug adverse effect, severe infection, or GVHD must be excluded as causes of peripheral blood cytopenias.

12.2 Definition of Response

12.2.1 Definition of Response: MDS/CMML

(A) *Complete Response*

- Bone marrow rating: Normal maturation of all cell lines, without morphologic significant dysplasia and with< 5% myeloblast.
- Peripheral blood rating: no peripheral blasts and no dysplasia.

(B) *Complete Response with normal blood counts*[37]

- Bone marrow rating: Normal maturation of all cell lines, without morphologic significant dysplasia and < 5% myeloblasts.
- Peripheral blood rating: Hb >11 g/dL, neutrophils ≥1500/mm^3, platelets >100 000/mm^3, blast 0%, no dysplasia.

(C) *Progressive Disease*

- Any evidence by morphologic or flow cytometric evaluation of the bone marrow aspirate of an incremental increase in 5% blasts (e.g. if a patient is accrued with 9% blasts, HCT failure would be ≥14% blasts).

12.2.2 Definition of Response: Agnogenic Myeloid Metaplasia[38]

(A) *Complete Response*

In patients with marrow fibrosis:

- Achievement of ≥95% donor chimerism in the bone marrow, and:
- evidence of regression of fibrosis as determined by sequential bone marrow biopsies (however residual fibrosis may be present).

In patients with myelodysplastic features or with leukemic transformation:

- achievement of ≥95% donor chimerism in the marrow, and:
- regression of marrow fibrosis, and:

- absence of leukemic blasts, and:
- disappearance of dysplastic changes.

(B) *Progressive Disease*

- Any evidence of blastic transformation.

12.2.3 Definition of Response: Atypical CML

(A) *Complete Response*

- normal peripheral blood counts and leukocyte differential OR achievement of ≥95% donor chimerism in the bone marrow, and:
- resolution of pretreatment cytogenetic abnormality, and:
- Normal maturation of all cell lines, without morphologic significant dysplasia, and:
- < 5% myeloblasts.

(B) *Progressive Disease*

- Any evidence of blastic transformation.

12.2.4 Definition of Response: Polycythemia Vera and Essential Thrombocythemia

(A) *Complete Response*

- Hematocrit ≤ 45% in the absence of phlebotomy, normal platelet count (< 400,000/ml) or achievement of ≥95% donor chimerism in the bone marrow.

(B) *Progressive Disease*

- Erythrocytosis, thrombocytosis, or evidence of leukemic transformation.

12.2.5 Definition of Response: PNH

(A) *Complete Response*

- Greater than 95% of the red blood cells not expressing the anchor proteins (documented by flow cytometry) in the absence of transfusions or achievement of ≥95% donor chimerism in the bone marrow.
- No clinical evidence of hemolysis related to PNH

13. Patient and donor Evaluations

A. Donor

Related Donor

Related donors will undergo standard evaluation for allogeneic stem cell donation, including:

1. Complete history and physical examination.
2. Lab tests: CBC with reticulocytes and platelet counts, serum sodium, potassium, chloride, CO2, BUN, creatinine, uric acid, LDH, calcium, magnesium, phosphate, alkaline phosphatase, AST, ALT, hepatitis screen, CMV, syphilis, HIV and HTLV I serologies and ABO Rh blood typing. If the donor has antibodies against red cell antigens of the recipient, the titers will be determined. Cytotoxic crossmatch between patient and donor (HLA Laboratory) will be performed.
3. CBC prior and after leukapheresis collection, plus daily while on G-CSF and if clinically indicated.
4. A re-evaluation in the OPD after apheresis is completed.
5. Attainment of a heparinized blood sample for subsequent determination of the host or donor origin of relapse to the cytogenetics lab or the clinical immunogenetics lab as outlined in section 13.B.1.e.
6. For females of child bearing age, serum pregnancy qualitative [PGSTAT] within 72 hours prior to initial dose of filgrastim (G-CSF). Results must be available prior to filgrastim (G-CSF) dose.

Unrelated Donor

Unrelated donors will undergo evaluation for allogeneic hematopoietic cell donation at the collection center by NMDP standard. The attending physician of the collection center will review the results of the donor evaluation. Evaluations typically include:

1. Complete history and physical examination.
2. Lab tests: CBC with reticulocytes and platelet counts, chemistries and LFT's, hepatitis screen, CMV, syphilis, HIV and HTLV I serologies and ABO Rh blood typing. If the donor has antibodies against red cell antigens of the recipient, the titers will be determined. Cytotoxic crossmatch between patient and donor (HLA Laboratory) will be performed.
3. No placement of a central line is necessary for G-CSF stimulated PBSC collection unless it is determined that the donor has poor venous access. If necessary, a temporary apheresis (e.g. Mahurkar) catheter will be placed at the time of leukapheresis.
4. CBC will be checked prior to and after leukapheresis collection, and daily while on G-CSF. CBCs will be checked thereafter if clinically indicated.
5. The donor will be reevaluated the day after the apheresis is completed.

B. Patient Evaluation

1. Patient Pre-transplant Evaluation for All Diseases

1. History: Complete history with full details of the patient's prior treatment and response.
2. Careful physical exam with determination of Karnofsky Performance Score (**Appendix B**) or Lansky Play-Performance Score (**Appendix C**) and HCT-comorbidity index score (**Appendix Q**).
3. Chest x-ray, P and lateral views.
4. ECHO or MUGA for patients > 50 years of age, or history of cardiac disease or anthracycline exposure.
5. Pulmonary function test with corrected DLCO.
6. CBC/differential, creatinine, BUN, uric acid, SMA-12, total bilirubin, alkaline phosphatase, AST, ABO/Rh typing, hepatitis screen, CMV and toxoplasma serology, and anti-HIV serology.
7. For CNS Disease: Please refer to **Appendix N** for recommendations for intrathecal diagnostic evaluation and prophylaxis for specific malignant diseases. If patients undergo intrathecal diagnostic evaluation, cerebral fluid should be sent for cell count and differential, cytospin, cytology, total protein, and glucose.

Additionally, see Table 9 for disease specific pre-transplant evaluations.

Table 9: Disease-Specific Pre-Transplant Evaluations

Note: All bone marrow aspirates and biopsies are **unilateral** and must be collected within **21 days** of treatment. See Tables 10 and 11 for post-transplant evaluations and additional lab instructions.

Specimen / Test / Imaging	Clinical / Research	Comment
Bone marrow aspirate **see biopsy*		
Pathology	Clinical	
Flow Cytometry	Clinical	
Cytogenetics	Clinical	
FISH for clonal abnormalities	Clinical	
Bone marrow biopsy		
Pathology	Clinical	
Peripheral Blood		
Storage for chimerism analysis	Clinical	
LDH	Clinical	
Flow Cytometry for PNH Panel	Clinical	**Only PNH*

2. Patient Post-transplant Evaluation

1. See Table 10 for disease specific post-transplant evaluation on Day +28, 56, 84, etc. This is a recommended evaluation schedule.

Additionally, include the following for all diseases:

2. CBC three times a week, or more often if clinically indicated, from day 0 until day +28, and twice weekly until 2 months post-transplant or later if clinically indicated
3. Electrolytes (sodium, potassium, chloride, CO_2, glucose, BUN, creatinine, calcium, magnesium, phosphorus, albumin) three times a week and liver function tests (ALT, AST, ALK, total bilirubin, direct bilirubin, total protein, albumin, LD) two times a week until day +28 and then every week.
4. Evaluate at Day +84:

   A patient with an uncomplicated unrelated HCT would be discharged after the day +84 workup and screening for chronic GVHD are completed and analyzed. Since the patient may be discharged prior to starting CSP taper, instructions should be provided for preventing and detecting GVHD as per standard practice of collaborating institution.

GVHD evaluation guidelines are as follows:

- History and physical exam.
- CBC/differential, serum IgG, total bilirubin, alkaline phosphatase, ALT and AST.
- Skin biopsy.
- Schirmer´s tear test.
- Pulmonary function test.
- Oral exam.
- Dietician assessment.
- Gynecological assessment (adult female).

See **Section 14.F** for diagnosis and treatment guidelines of acute and chronic GVHD.

5. Patients should be assessed for the need of IVIG monitoring and replacement therapy per Institutional Guidelines

Table 10: Post-Transplant Evaluation

This is a recommended evaluation schedule. See Table 9 for pre-transplant evaluations. Additional lab instructions in Table 11.

Disease	Specimen/ Test/ Imaging	Clinical/ Research	Comment	Days				Years		Annual x 5 years
				28	56	84	180	1	1.5	
CMML	**BM aspirate** **see biopsy* ** If CR documented at one year, and there is recovery of normal blood counts, bone marrow after one year may be obtained based on clinical judgment									
	Chimerism	Clinical				x		x		
	Pathology	Clinical		x	x	x	x	x	x	x
	Flow Cytometry	Clinical		x	x	x	x	x	x	x
	Cytogenetics	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	FISH for t(5:12) and other clonal abnormalities	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	BM biopsy									
	Pathology	Clinical	*For pts. with evidence or history of myelofibrosis	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	Peripheral blood									
	Chimerism (CD3+)	Clinical	*Days 56 and 180 only if <50% on day 28	x	*See comment	x	*See comment	x		
	Chimerism (CD33+)	Clinical				x				
	Chimerism (NK CD56+)	Clinical	Optional for outside institutions	x						
	GVHD evaluation	Clinical	See text for details			x				

Disease	Specimen/ Test/ Imaging	Clinical/ Research	Comment	Days				Years		Annual x 5 years
				28	56	84	180	1	1.5	
MDS (RAEB)	**BM aspirate** **see biopsy* ** If CR documented at one year, and there is recovery of normal blood counts, bone marrow after one year may be obtained based on clinical judgment									
	Chimerism	Clinical				x		x		
	Pathology	Clinical		x	x	x	x	x	x	x
	Flow Cytometry	Clinical		x	x	x	x	x	x	x
	Cytogenetics	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	FISH for del (5q), del (7q), trisomy 8, 11q23 (MLL), del (13q)	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	BM biopsy									
	Pathology	Clinical	*For pts. with evidence or history of myelofibrosis	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	Peripheral blood									
	Chimerism (CD3+)	Clinical	*Days 56 and 180 only if <50% on day 28	x	*See comment	x	*See comment	x		
	Chimerism (CD33+)	Clinical				x				
	Chimerism (NK CD56+)	Clinical	Optional for outside institutions	x						
	GVHD evaluation	Clinical	See text for details			x				

Disease	Specimen/ Test/ Imaging	Clinical/ Research	Comment	Days				Years		Annual x 5 years
				28	56	84	180	1	1.5	
MPD	**BM aspirate** **see biopsy* ** If CR documented at one year, and there is recovery of normal blood counts, bone marrow after one year may be obtained based on clinical judgment									
	Chimerism	Clinical				x		x		
	Pathology	Clinical		x	x	x	x	x	x	x
	Flow Cytometry	Clinical		x	x	x	x	x	x	x
	Cytogenetics	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	FISH for t(5:12) and other clonal abnormalities	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	BM biopsy									
	Pathology	Clinical	*For pts. with evidence or history of myelofibrosis	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	Peripheral blood									
	Chimerism (CD3+)	Clinical	*Days 56 and 180 only if <50% on day 28	x	*See comment	x	*See comment	x		
	Chimerism (CD33+)	Clinical				x				
	Chimerism (NK CD56+)	Clinical	Optional for outside institutions	x						
	GVHD evaluation	Clinical	See text for details			x				

Disease	Specimen/ Test/ Imaging	Clinical/ Research	Comment	Days				Years		Annual x 5 years
				28	56	84	180	1	1.5	
PNH	**BM aspirate** **see biopsy* ** If CR documented at one year, and there is recovery of normal blood counts, bone marrow after one year may be obtained based on clinical judgment									
	Chimerism	Clinical				x		x		
	Pathology	Clinical		x	x	x	x	x	x	x
	Cytogenetics	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	FISH for clonal abnormalities	Clinical	*If abnormal pre-transplant	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	BM biopsy									
	Pathology	Clinical	*For pts. with evidence or history of myelofibrosis	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment	*See comment
	Peripheral blood									
	Chimerism (CD3+)	Clinical	*Days 56 and 180 only if <50% on day 28	x	*See comment	x	*See comment	x		
	Chimerism (CD33+)	Clinical				x				
	Chimerism (NK CD56+)	Clinical	Optional for outside institutions	x						
	Flow Cytometry for PNH Panel	Clinical	*If abnormal pre-transplant	x	x	x	x	x	x	x
	GVHD evaluation	Clinical	See text for details			x				

Table 11: Additional Lab Instructions

Note: All bone marrow tests are done on aspirate unless specifically identified as biopsy. All instructions apply to both pre- and post-transplant evaluations unless identified otherwise.

Off-site providers may use local facilities for the tests.

Volumes represent desired amounts.

Specimen / Test	Type	Instructions	Lab Name	Contact Information
Bone marrow				
Chimerism	Clinical	1-3mL bone marrow in green-top tube	Clinical Immunogenetics Lab	Seattle Cancer Care Alliance (206) 288-7700
Pathology (*aspirate*)	Clinical	2mL bone marrow in EDTA/ formalin	SCCA Pathology Lab	Seattle Cancer Care Alliance (206) 288-1355
Pathology (*biopsy*)	Clinical	1cm bone marrow in formalin **OR** mounted in paraffin	SCCA Pathology Lab	Seattle Cancer Care Alliance (206) 288-1355
Flow Cytometry	Clinical	2mL bone marrow in green-top tube	UW Hematopathology Lab	Seattle Cancer Care Alliance (206) 288-7060
Cytogenetics	Clinical	3mL bone marrow in green-top tube	SCCA Cytogenetics Lab	Seattle Cancer Care Alliance (206) 288-1390
FISH	Clinical	2mL bone marrow in green-top tube	SCCA Cytogenetics Lab	Seattle Cancer Care Alliance (206) 288-1390
Peripheral blood				
Chimerism (CD3+), (CD33+) NK(CD56+)	Clinical	10mL blood in green-top tube for Flow sorting, then to CIL	UW Hematopathology Lab, routed to Clinical Immunogenetics Lab	Mailstop G7-800 825 Eastlake Ave, East Seattle, WA 98109 (206) 288-7060
LDH	Clinical	3mL blood in red-top tube	SCCA Alliance Lab	Seattle Cancer Care Alliance (206) 288-2057

Outside institutions may use VNTR analysis (sex- matched transplants) or sex chromosome FISH-analysis (sex-mismatched transplants) for chimerism analysis.

14. Drugs and Toxicities

A. Toxicity: For the purposes of this protocol, toxicity will be graded using the modified NCI common toxicity scale (**Appendix P**).

B. TBI: TBI will be given in one 300 cGy (or 400 cGy in level 2 and 450 cGy in level 3) fraction from linear accelerator at a rate of 6-7 cGy/min. Dosimetry calculations are performed by the radiation therapist. At the 300 cGy, side effects are not expected. Nevertheless, there may be fever, alopecia, parotitis, diarrhea, reversible skin pigmentation, mucositis and late effects including cataract formation, growth retardation, pulmonary damage, carcinogenesis, and sterilization. Those side effects might be more frequently seen in patients given 400 cGy or 450 cGy.

C. CSP: See section **11.F.a** for information about administration and dosage adjustments. Side effects are generally reversible and may include renal insufficiency and failure, hypomagnesemia, paresthesias, tremor, seizures, visual disturbances, paresis, disorientation, depression, confusion, somnolence, coma, nausea, hypertension, hemolytic-uremic syndrome, hyperglycemia, gynecomastia, and hypertrichosis.

D. MMF: See section **11.F.b** for information about administration and dosage adjustments.

Precautions: MMF has been studied extensively among patients after nonmyeloablative HCT. Previous clinical studies in patients after allografting suggest that the principal adverse reactions

associated with the administration of MMF include nausea, vomiting, neutropenia, diarrhea, and on one occasion bloody diarrhea. In the setting of marrow transplantation, several etiologic factors may contribute to alterations in gastrointestinal and hematologic parameters. MMF has an increased incidence of digestive system adverse events, including GI tract ulceration, and hemorrhage (3% of patients receiving MMF). GI tract perforations have rarely been observed. Most patients in these studies were also on other drugs known to be associated with these complications. Up to 2% of patients receiving MMF for prevention of rejection developed severe neutropenia (ANC <500). The development of neutropenia may be related to MMF itself, concomitant medications, viral infections or some combination of these causes. MMF dose adjustments will be made if clinically indicated if in the opinion of the attending physician, no other cause is thought to be responsible for the abnormality. These adjustments should be discussed with the principal investigator and documented in the medical records and the clinical reporting form (CRF).

E. Fludarabine: The doses of fludarabine used in this protocol are nonmyeloablative, but do cause significant immunosuppression. Fludarabine can lower the white blood cell count, in particular the CD4+ T-cells. The immunosuppression observed with the use of fludarabine increases the risk of infection, which can be life threatening.

F. GVHD: After nonmyeloablative conditioning, the incidences of grades II, III and IV acute GVHD were 33%, 10% and 5%, respectively, in MRD recipients, and 42%, 9% and 3%, respectively, in URD recipients (Table 1). Acute GVHD has been readily controlled in most patients with high dose corticosteroids, but PUVA (psoralen activated ultraviolet light) has been required on occasion. Chronic extensive GVHD has occurred in 43% and 45% of MRD and URD recipients, respectively.

1. Diagnosis: Skin involvement will be assessed by biopsy with percentage of body surface area involved recorded. GI symptoms suspicious for GVHD will be evaluated by biopsy as indicated. Acute GVHD and chronic GVHD will be graded according to established criteria (**Appendix F and G**).

2. Recommended Treatment:
   a. Patients developing acute GVHD ≥ grade II off immunosuppression or while on a CSP taper:
      1). CSP 5mg/kg PO q12hrs. If there is concern of GI absorption use IV route (1.5mg/kg q12hrs).
      2). Prednisone (2mg/kg/day) is to be added if there is no response by 72 hours or progression of GVHD during the 24 hours after the start of CSP 6.0mg/kg POq12hrs. Patients who respond to steroids after 10 to 14 days of treatment, should begin a 6-week steroid taper.
      3). Patients may also be eligible for institutional trials of GVHD therapy.
   b. Patients who develop acute GVHD ≥ grade II prior to day +100:
      1). Patients who develop acute GVHD ≥ grade II while on full dose MMF should receive prednisone (2mg/kg/day) or intravenous equivalent. When steroids are tapered to 0.5mg/kg PO QD then an MMF taper should be initiated. In the absence of a GVHD flare, the MMF and prednisone tapers should continue until completion. If nausea and/or vomiting prevent the oral administration of MMF, MMF should be administered intravenously at 15mg/kg q12hrs IV, or q8hrs if prior to Day + 28. Patients who respond to steroids after 10 to 14 days of treatment, should begin a 6-week steroid taper.

2). Patients who develop acute GVHD ≥ grade II while not receiving full dose MMF should receive prednisone (2mg/kg/day) or intravenous equivalent. MMF need not be restarted at full dose.
3). Patients may also be eligible for institutional trials of GVHD therapy.

c. Patients with clinical extensive chronic GVHD: CSP 5.0mg/kg PO q12hrs and prednisone 1mg/kg QD or eligible protocols at the time. The patient should receive antibiotic prophylaxis with daily double strength Bactrim.
d. Patients off immunosuppression who develop concurrent manifestations of GVHD that satisfy criteria for acute GVHD ≥ grade II (e.g. erythematous rash, diarrhea, hyperbilirubinemia) **and** have stigmates of clinical extensive chronic GVHD (e.g. lichenoid oral changes, ocular sicca, scleroderma, bronchiolitis obliterans, contractures), should receive prolonged immunosuppressive therapy similar to that for clinical extensive chronic GVHD.

G. Myelosuppression

Grade IV myelosuppression will be defined as a decrease in ANC to ≤500/μL and/or platelet count to ≤ 20,000/μL. If myelosuppression occurs, a bone marrow aspirate and biopsy should be performed to exclude disease progression. Samples should be sent for chimerism analysis by a DNA-based assay that compares the profile of amplified fragment length polymorphisms (ampFLP) (or FISH studies or VNTR). Myelosuppression may occur in this patient population for a number of reasons such as direct toxic effect of drugs (MMF, ganciclovir etc.), rejection, relapse or after DLI.

Patients with myelosuppression may be managed as follows:

1. Suspected MMF toxicity: refer to sections **11.F.b.4** Guidelines for MMF dose adjustment above for management recommendations.
2. Suspected ganciclovir toxicity: consider changing to foscarnet.
3. Patients who are > 21 days after HCT with a hypoplastic marrow and an ANC of ≤750/μL may receive G-CSF.
4. Thrombocytopenic patients will receive platelet transfusion as per standard care.

15. Records

Clinical records will be maintained as confidentially as possible by all collaborating institutions. Collection of Case Report Forms (CRF) at standard intervals is the primary method of collecting data from collaborating centers. Clinical Statistics at FHCRC maintains a patient database to allow storage and retrieval of patient data collected from a wide variety of sources. The principal investigator will ensure that data collected conform to all established guidelines for coding collection, key entry and verification. These data are then entered into a secure dedicated database operated by a data manager. Any publication or presentation will refer to patients by a unique patient number and not by name to assure patient confidentiality. The licensed medical records department, affiliated with the institution where the patient receives medical care, maintains all original inpatient and outpatient chart documents.

At the FHCRC, patient research files are kept in a locked room. They are maintained by the FHCRC data collection staff that is supervised by an A.R.T. Access is restricted to personnel authorized by the Division of Clinical Research.

16. Statistical Consideration and Termination of Study

The primary objective of this protocol is to evaluate whether a more intense but still nonmyeloablative conditioning regimen can reduce the combined rate of graft rejection and disease progression (HCT failure) in this group of MDS and CMML patients, while maintaining an acceptable rate of nonrelapse mortality. Previous protocols with standard nonmyeloablative conditioning regimens have yielded HCT failure rates of 0.30-0.60. For purposes of this protocol HCT failure will be defined as graft rejection (defined as < 5% donor T-cell chimerism) or disease progression (see table 8 page 25) within 200 days of transplant.

Dose escalation will be carried out independently in two groups of patients:
Arm A – patients with MPD, MDS-RA/RARS, or PNH
Arm B – patients with MDS-RAEB or CMML

In each arm, up to 24 patients will be accrued to each TBI dose level, in groups of 6 patients, with an escalation rule triggered for excessive HCT failure. If 24 patients are successfully enrolled at a TBI dose level without triggering the escalation rule for HCT failure (or other stopping rule), then that dose level will be considered a success and accrual will be closed for that arm. The proposed TBI dose levels are:

Dose Level 1) 300 cGy TBI + fludarabine 30/m^2/day IV x 3 days
Dose Level 2) 400 cGy TBI + fludarabine 30/m^2/day IV x 3 days
Dose Level 3) 450 cGy TBI + fludarabine 30/m^2/day IV x 3 days

Dose escalation rules will be imposed for:

- HCT failure >20% at day 200 on **Arm A** or **Arm B**

Stopping rules will be imposed for:

- NRM of > 25% at day 200 on **Arm A**
- NRM of > 35% at day 200 on **Arm B**

Enrollment to a dose level will occur in groups of 6 patients. Escalation to the next dose level will occur if there exists reasonable evidence that the true rate of HCT failure exceeds 0.20. Reasonable evidence will be taken to mean that the lower bound of a one-sided 80% confidence interval for the true rate is greater than 0.20. Operationally, this rule will be triggered if 3 or more of 6, 5 or more of 12, 6 or more of 18, or 7 or more of 24 patients experience HCT failure. Accrual may continue pending evaluability of enrolled patients; however, at any point the escalation rule is triggered the outcome of subsequently enrolled patients will not override it. The operating characteristics of this escalation rule are provided in the table below.

Stopping rules will also be applied in each arm for non-relapse mortality within 200 days of transplant. Accrual to a dose level will stop if there exist reasonable evidence that the true rate of nonrelapse mortality exceeds 0.25. Reasonable evidence will be taken to mean that the lower bound of a one-sided 80% confidence interval for the true rate is greater than 0.25. Operationally, this rule will be triggered if 3 or more of 6, 5 or more of 12, 7 or more of 18, or 9 or more of 24 patients experience NRM. Accrual may continue pending evaluability of enrolled patients; however, at any point the stopping rule is triggered the outcome of subsequently enrolled patients will not override it. If the stopping rule is triggered, then dose escalation is not permitted and consequently accrual to that arm of the protocol will be closed. The operating characteristics of this escalation rule are provided in the table below.

True rate of HCT failure	Probability of escalation*	True rate of nonrelapse mortality	Probability of stopping*
0.25	47%	0.30	46%
0.30	67%	0.35	64%
0.35	82%	0.40	79%
0.40	92%	0.45	90%

*based on 10,000 Monte Carlo simulations

Additional accrual to Arm A
If ARM A completes accrual of 24 patients at a dose level, up to 12 additional patients may be accrued at the same dose level while accrual to Arm B continues. Stopping rules for non-relapse mortality will continue to be monitored. Additional stopping points will be 10 NRM deaths in 30 or fewer patients and 12 NRM deaths in 36 or fewer patients.

Revised stopping rule for Arm B
The initial cohort of 6 patients enrolled into Arm B experienced 3 non-relapse deaths before day 200, triggering the original stopping rule. Of the 5 patients evaluable for disease response (one patient died day 19 from Human metapneumovirus and, thus, was not evaluable), all 5 engrafted with day 28 CD3 chimerism ranging from 64-89% and 4 of 5 patients achieved a complete remission. After reviewing recent data with similar patient populations (26 patients with CMML/RAEB), it was determined that the original threshold rate of 0.25 for non-relapse mortality was too conservative. Although a rate of 0.25 accurately reflects the observed NRM in these patients, it does not accommodate the increase in NRM that might occur if better engraftment and disease control is successfully achieved, thus placing more patients at risk for NRM. Specifically, 17 patients (65%) relapsed or progressed 108 (28-1585) days after HCT, while 5 (19%) rejected their grafts 80 (14-127) days after HCT. All patients with graft rejection eventually progressed. Consequently, accrual to Arm B will be restarted with a revised stopping rule based on a threshold rate of 0.35 for non-relapse mortality. The outcome of the initial 6 patients enrolled at the first dose level in Arm B will carry forward and be included in the evaluation of the stopping rule after restarting. Escalation and stopping rules for Arm A are unchanged.

The revised stopping rule for Arm B will be triggered if 4 or more of 6, 7 or more of 12, 9 or more of 18, or 11 or more of 24 patients experience NRM before day 200. The operating characteristics of this stopping rule are provided in the table below.

True rate of HCT failure	Probability of escalation*	True rate of nonrelapse mortality	Probability of stopping*
0.25	47%	0.40	43% (50%)
0.30	67%	0.45	63% (66%)
0.35	82%	0.50	77% (80%)
0.40	92%	0.55	90% (90%)

* based on 10,000 Monte Carlo simulations. The percent in parentheses is the conditional probability of stopping in the restarted Arm B, given that 3 of 6 patients already have experienced NRM by day 200. The other percent applies if the stopping rule is applied to a higher dose level within Arm B.

If a cohort of 24 patients is completed without excessive HCT failure, then no more than 6 HCT failures will have occurred. Nominally this would mean that we could be 80% confident that the true rate of HCT

failure was less than 35%; however, because of the continuous monitoring and escalation rule for HCT failure, the final estimated failure rate will be an underestimate of the true failure rate.

Secondary endpoints to be evaluated will include:

- the rate of relapse/progression in patients with MPD or MDS-RA and those with CMML or MDS-RAEB.
- the probability of PFS in patients with MPD or MDS-RA and those with CMML or MDS-RAEB.
- the kinetics of donor engraftment.
- the incidence of infections.

17. Data and Safety Monitoring Plan

A. FHCRC Protocol 2056 Data and Safety Monitoring Plan

1. Monitoring the progress of trials and the safety of participants

   Protocol 2056 is a multi-institutional clinical trial that is monitored by the principal investigator (PI), Dr. Sandmaier, a Data Safety and Monitoring Board (DSMB), the Data and Safety Monitoring Committee (DSMC) and the Institutional Review Board (IRB). The PI reviews outcome data with the protocol mentor for each individual patient at a minimum of 3 months after unrelated donor HCT and the updated data are presented at Mixed Chimerism Meetings (includes co-investigators).

   Please see **Appendix I** for definitions of adverse events, serious adverse events (SAE) and serious and unexpected events as well as mechanisms for reporting these events. SAEs are reported to the trial coordinator. The trial coordinators at collaborating centers or the local PIs will report SAEs within 10 days to the coordinating center (FHCRC). The SAEs report is reviewed by Dr. Sandmaier. If the SAE meets the FHCRC expedited criteria for reporting then an official signed report is submitted to the FHCRC Institutional Review Office (IRO). All deaths, regardless of the cause, are reported to the IRB. Protocol 2056 has a dedicated independent DSMB responsible for monitoring patient safety on this clinical trial. The DSMB will meet at six-month intervals for this protocol and all outcome data is reviewed including all adverse events and SAEs reported to the coordinating center (FHCRC) along with those officially reported to the FHCRC IRO. The DSMB confirms whether the trial has met any stopping rules and reviews any patient safety problems necessitating discontinuation of the trial. A report from the DSMB is submitted to the FHCRC IRB as well as the trial coordinators/local PIs of this protocol. The DSMB will discontinue the review of outcomes when this protocol is closed to accrual. Furthermore, the FHCRC also has a DSMC that reviews the progress of the protocol with respect to the monitoring plan at the time of each annual renewal. As with initial review, annual IRB review and approval is also required.

   Flow of information concerning clinical trial participants originates with the clinicians and nurses in the clinic or referring clinicians at other institutions and is transmitted to the trial coordinator and data manager. At the FHCRC, health care providers and rotating attending physicians assess patients and record their observations regarding toxicity and response outcomes in the medical record. This documentation is extracted by the study nurse at 100 days after HCT via chart review and collection of copies of source documents and entered into a hard copy or electronic Case Report Form (CRF) by approximately day +140 post transplant. The PI reviews the official CRF and primary source documents. When the CRFs are verified, they are signed by the PI. Thus, multiple health care providers provide independent observations and participate in monitoring this trial. The PI may be a clinician for some patients entered on this trial. However, assessments are the sum total of the primary health care provider (fellow or physician assistant), floor or outpatient nurse and the PI or other

attending clinician involved with the patient averting possible conflict of interest having the PI as the attending clinician for protocol patients. If determination of adverse events is controversial, co-investigators will convene on an ad hoc basis as necessary to review the primary data and render a decision.

Protocol 2056 will be a multi-institutional protocol. All collaborating centers sign an agreement with the FHCRC stating that data generated from patients from the protocol will be reported accurately in a timely manner to the FHCRC. All centers have an IRB that reviews the protocol and that the local PIs contact when an adverse event on the protocol occurs. Most of the centers have internal auditing mechanisms that assure accurate assessment of clinical outcomes. Clinical outcome data are summarized and transmitted from collaborating centers as CRFs. When possible, primary source documents regarding patient outcomes are collected with patients' names removed and replaced by Unique Patient Numbers (UPNs). The CRFs are generated from the collaborating centers at defined time points (100 days, 6 months, and yearly). They are reviewed by local PI, signed, and sent for encoding in the database.

2. Plans for assuring compliance with requirements regarding the reporting of Serious Adverse Events SAEs

   The adverse event reporting in this multi-institutional clinical trial will follow an adapted version of the FHCRC Guidelines for SAE reporting. These guidelines (attached in **Appendix I**.) detail the expedited reporting requirements, definitions of particular events. All SAEs that meet expedited criteria are reported to the IRO within 10 days by the investigator, trial coordinator, or research nurse upon learning of the event. A completed SAE report form, signed by the PI, must be received by the IRO within 10 calendar days. The PI's protocol mentor reviews all SAEs and annual reports at the time of submission. For patients being cared for at the FHCRC, health care providers communicate with the PI, trial coordinator or research nurses as events occur triggering subsequent reporting. For patients not being cared for at FHCRC the outside facilities communicate with the PI, trial coordinator, or research nurse for these reporting purposes. All other deaths and expected serious adverse events are reported to the IRB at the time of annual renewal and at the biannual mixed chimerism meeting. The PI for a study is responsible for this reporting and the IRO assures adverse event reporting on an annual basis. The PI in the annual application for grant continuation will summarize reports of toxicities. Furthermore, an additional safeguard for adverse event analysis and reporting in this protocol is provided by stopping rules and interim analysis after every 10th patient is enrolled. All collaborating PIs have fulfilled all NIH requirements for training in human subjects protection.

3. Plans for assuring that any action resulting in a temporary or permanent suspension of an NCI-funded clinical trial is reported to the NCI grant program director responsible for the grant

   This clinical research trial uses commercial agents and there is no associated Investigational New Drug (IND) or Investigational Device Exemption (IDE). Any temporary or permanent suspension, as determined by the PI, IRB, or DSMC, of this clinical research trial will be reported to the NCI grant program director by the PI.

4. Plans for assuring data accuracy and protocol compliance
   Collaborating sites send signed consents, eligibility forms, and CRFs with source documents demonstrating eligibility, treatment, and serious adverse events (if applicable) to the study staff. These are reviewed for eligibility, adherence to the protocol, accuracy, and completeness by the study staff. Queries are sent to the collaborating investigators if CRFs are inaccurate or incomplete.

   The study is monitored under the FHCRC Monitoring Plan. The FHCRC Data and Safety Monitoring Plan details the full scope and extent of monitoring and provides for immediate action in the event of the discovery of major deviations.

18. Targeted / Planned Enrollment

TARGETED / PLANNED ENROLLMENT: Number of Subjects			
	Sex / Gender		
Ethnic Category	**Females**	**Males**	**Total**
Hispanic or Latino	2	2	4
Not Hispanic or Latino	58	82	140
Ethnic Category Total of All Subjects*	60	84	144
Racial Categories			
American Indian / Alaska Native	2	2	4
Asian	2	2	4
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	2	4	6
White	54	76	130
Racial Categories: Total of All Subjects*	60	84	144

**The "Ethnic Category Total of All Subjects" must be equal to the "Racial Categories Total of All Subjects."*

Reference List

(1) Scott B, Deeg HJ. Hemopoietic cell transplantation as curative therapy of myelodysplastic syndromes and myeloproliferative disorders. In: Tefferi A, ed. Ballieres Best Practice and Research. 9999:(in press).

(2) McSweeney PA, Niederwieser D, Shizuru JA et al. Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. Blood. 2001;97:3390-3400.

(3) Niederwieser D, Maris M, Shizuru JA et al. Low-dose total body irradiation (TBI) and fludarabine followed by hematopoietic cell transplantation (HCT) from HLA-matched or mismatched unrelated donors and postgrafting immunosuppression with cyclosporine and mycophenolate mofetil (MMF) can induce durable complete chimerism and sustained remissions in patients with hematological diseases. Blood. 2003;101:1620-1629.

(4) Maris MB, Niederwieser D, Sandmaier BM et al. HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative conditioning for patients with hematologic malignancies. Blood. 2003;102:2021-2030.

(5) Sandmaier BM, Maris M, Maloney DG et al. Low-dose total body irradiation (TBI) conditioning for hematopoietic cell transplants (HCT) from HLA-matched related (MRD) and unrelated (URD) donors for patients with hematologic malignancies: a five-year experience [abstract]. Blood. 2003;102 (Part 1):78a-79a, #264.

(6) Baron F, Maris MB, Sandmaier BM et al. Graft-versus-tumor effects after allogeneic hematopoietic cell transplantation with nonmyeloablative conditioning. J Clin Oncol. 2005;23:1993-2003.

(7) Scott BL, Maris M, Sandmaier B et al. Myeloablative versus nonmyeloablative hemopoietic cell transplantation (HCT) for patients with myelodysplasia (MDS) or AML with multinlineage dysplasia following MDS (tAML) [abstract]. Blood. 2004;104 (Part 1):638a, #2320.

(8) Storb R, Yu C, Wagner JL et al. Stable mixed hematopoietic chimerism in DLA-identical littermate dogs given sublethal total body irradiation before and pharmacological immunosuppression after marrow transplantation. Blood. 1997;89:3048-3054.

(9) Sandmaier BM, Storb R. Nonmyeloablative therapy and hematopoietic cell transplantation for hematologic disorders. In: Blume KG, Forman SJ, Appelbaum FR, eds. Thomas' Hematopoietic Cell Transplantation. Oxford, UK: Blackwell Publishing Ltd.; 2004:1164-1176.

(10) Burroughs L, Leisenring W, Maloney DG et al. Extending postgrafting cyclosporine does not decrease the risk of graft-versus-host disease after nonmyeloablative hematopoietic cell transplantation [abstract]. Blood. 2003;102 (Part 1):242a-243a, #852.

(11) Baron F, Maris MB, Storer BE et al. High doses of transplanted $CD34^+$ cells are associated with rapid T-cell engraftment and lessened risk of graft rejection, but not more graft-versus-host disease after nonmyeloablative conditioning and unrelated hematopoietic cell transplantation. Leukemia. 2005;19:822-828.

(12) Maris MB, Sandmaier BM, Storer B et al. Unrelated donor peripheral blood stem cell (PBSC) transplantation using nonmyeloablative conditioning and mycophenolate mofetil (MMF) TID results in high engraftment rates [abstract]. Blood. 2004;104 (Part 1):503a, #1818.

(13) Mielcarek M, Martin PJ, Leisenring W et al. Graft-versus-host disease after nonmyeloablative versus conventional hematopoietic stem cell transplantation. Blood. 2003;102:756-762.

(14) Sorror ML, Maris MB, Storer B et al. Comparing morbidity and mortality of HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative and myeloablative conditioning: influence of pretransplant comorbidities. Blood. 2004;104:961-968.

(15) Diaconescu R, Flowers CR, Storer B et al. Morbidity and mortality with nonmyeloablative compared to myeloablative conditioning before hematopoietic cell transplantation from HLA matched related donors. Blood. 2004;104:1550-1558.

(16) Ho AYL, Pagliuca A, Kenyon M et al. Reduced-intensity allogeneic hematopoietic stem cell transplantation for myelodysplastic syndrome and acute myeloid leukemia with multilineage dysplasia using fludarabine, busulphan and alemtuzumab (FBC) conditioning. Blood. 2004;104:1616-1623.

(17) Bornhauser M, Storer B, Slattery JT et al. Conditioning with fludarabine and targeted busulfan before transplantation of allogeneic hematopoietic stem cells [abstract]. Blood. 2002;100 (Part 1):213a, #799.

(18) Kroger N, Bornhauser M, Ehninger G et al. Allogeneic stem cell transplantation after a fludarabine/busulfan-based reduced-intensity conditioning in patients with myelodysplastic syndrome or secondary acute myeloid leukemia. Ann Hematol. 2003;82:336-342.

(19) de Lima M, Anagnostopoulos A, Munsell M et al. Nonablative versus reduced-intensity conditioning regimens in the treatment of acute myeloid leukemia and high-risk myelodysplastic syndrome: dose is relevant for long-term disease control after allogeneic hematopoietic stem cell transplantation. Blood. 2004;104:865-872.

(20) Appelbaum FR. Dose intensity and the toxicity and efficacy of allogeneic hematopoietic cell transplantation (Keynote Address). Leukemia. 2005;19:171-175.

(21) Clift RA, Buckner CD, Appelbaum FR et al. Allogeneic marrow transplantation in patients with acute myeloid leukemia in first remission: A randomized trial of two irradiation regimens. Blood. 1990;76:1867-1871.

(22) Clift RA, Buckner CD, Appelbaum FR et al. Allogeneic marrow transplantation in patients with chronic myeloid leukemia in the chronic phase: A randomized trial of two irradiation regimens. Blood. 1991;77:1660-1665.

(23) Storb R, Raff RF, Appelbaum FR et al. What radiation dose for DLA-identical canine marrow grafts? Blood. 1988;72:1300-1304.

(24) Yu C, Storb R, Mathey B et al. DLA-identical bone marrow grafts after low-dose total body irradiation: Effects of high-dose corticosteroids and cyclosporine on engraftment. Blood. 1995;86:4376-4381.

(25) Storb R, Yu C, Wagner JL et al. Stable mixed hematopoietic chimerism in DLA-identical littermate dogs given sublethal total body irradiation before and pharmacological immunosuppression after marrow transplantation. Blood. 1997;89:3048-3054.

(26) Hogan WJ, Little M-T, Zellmer E et al. Postgrafting immunosuppression with sirolimus and cyclosporine facilitates stable mixed hematopoietic chimerism in dogs given sublethal total body irradiation before marrow transplantation from DLA-identical littermates. Biol Blood Marrow Transplant. 2003;9:489-495.

(27) Storb R, Raff RF, Appelbaum FR et al. DLA-identical bone marrow grafts after low-dose total body irradiation: the effect of canine recombinant hematopoietic growth factors. Blood. 1994;84:3558-3566.

(28) Girgis M, Hallemeier C, Blum W et al. Chimerism and clinical outcomes of 110 unrelated donor bone marrow transplants who underwent conditioning with low-dose, single-exposure total body irradiation and cyclophosphamide. Blood. 2005;105:3035-3041.

(29) Rowley MJ, Leach DR, Warner GA, Heller CG. Effect of graded doses of ionizing radiation on the human testis. Radiat Res. 1974;59:665-678.

(30) Sanders JE. Growth and development after hematopoietic cell transplantation. In: Blume KG, Forman SJ, Appelbaum FR, eds. Thomas' Hematopoietic Cell Transplantation. Oxford, UK: Blackwell Publishing Ltd.; 2004:929-943.

(31). Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis. 1987;40:373-383.
Reference ID: 22434

(32). Diaconescu R, Flowers CR, Storer B, et al. Morbidity and mortality with nonmyeloablative compared to myeloablative conditioning before hematopoietic cell transplantation from HLA matched related donors. Blood. 2004;104:1550-1558.
Reference ID: 24498

(33). Sorror ML, Maris MB, Storer B, et al. Comparing morbidity and mortality of HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative and myeloablative conditioning: influence of pretransplant comorbidities. Blood. 2004;104:961-968.
Reference ID: 24482

(34) Sorror ML, Maris MB, Storb R, et al. Hematopoietic cell transplantation-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. Blood. 9999;prepublished online June 30, 2005; DOI 10.1182/blood-2005-05-2004-
Reference ID: 28079

(35) Baron F, Baker JE, Storb R et al. Kinetics of engraftment in patients with hematologic malignancies given allogeneic hematopoietic cell transplantation after nonmyeloablative conditioning. Blood. 2004;104:2254-2262.

(36) Salmon JP, Michaux S, Hermanne JP et al. Delayed massive immune hemolysis mediated by minor ABO incompatibility after allogeneic peripheral blood progenitor cell transplantation. Transfusion. 1999;39:824-827.

(37) Cheson BD, Bennett JM, Kantarjian H et al. Report of an international working group to standardize response criteria for myelodysplastic syndromes. Blood. 2000;96:3671-3674.

(38) Deeg HJ, Gooley TA, Flowers MED et al. Allogeneic hematopoietic stem cell transplantation for myelofibrosis. Blood. 2003;102:3912-3918.

(39) Panse JP, Heimfeld S, Guthrie KA, et al. Allogeneic peripheral blood stem cell graft composition affects early T-cell chimaerism and later clinical outcomes after nonmyeloablative conditioning. Br J Haematol. 2005;128:659-667.

(40) Cao TM, Shizuru JA, Wong RM, et al. Engraftment and survival following reduced-intensity allogeneic peripheral blood hematopoietic cell transplantation is affected by CD8+ T-cell dose. Blood. 2005;105:2300-2306.

(41) Baron F, Maris MB, Storer BE, et al. High doses of transplanted CD34+ cells are associated with rapid T-cell engraftment and lessened risk of graft rejection, but not more graft-versus-host disease after nonmyeloablative conditioning and unrelated hematopoietic cell transplantation. Leukemia. 2005;19:822-828.

APPENDICES - Table of Contents

Appendix A
ELIGIBILITY GUIDELINES
FOR DONOR PBSC APHERESIS FOR TRANSFUSION

Immunization	*Donor Eligibility*
Cholera	No wait
Diphtheria	No wait
Flu	24 hour wait
Gamma globulin (Immune serum globulin)	No wait unless for hepatitis
Hepatitis B vaccine	No wait unless given for hepatitis exposure
Measles (Rubella)	1 month wait
Mumps	2 week wait
Polio – Sabin (inj)	No wait
Plague	No wait
Rabies	1 year wait if given as treatment for bite. 2 week wait if given as prophylaxis (DMV's or zoo workers)
Smallpox	2 week wait
Tetanus toxoid	No wait
Typhoid	No wait
Typhus	No wait
Yellow Fever	2 week wait

Appendix B
KARNOFSKY PERFORMANCE STATUS SCALE

General	*Index*	*Specific Criteria*
Able to carry on normal activity; no special care needed	100	Normal, no complaints, no evidence of disease
	90	Able to carry on normal activity, minor signs or symptoms of disease
	80	Normal activity with effort, some signs or symptoms of disease
Unable to work, able to live at home and care for most personal needs, varying amount of assistance needed	70	Care for self, unable to carry on normal activity or to do work
	60	Requires occasional assistance from others but able to care for most needs
	50	Requires considerable assistance from others and frequent medical care
Unable to care for self, requires institutional or hospital care or equivalent; disease may be rapidly progressing	40	Disabled; requires special care and assistance
	30	Severely disabled, hospitalization indicated, death not imminent
	20	Very sick, hospitalization necessary, active supportive treatment necessary
	10	Moribund
	0	Dead

Appendix C
LANSKY PLAY-PERFORMANCE SCALE
(for use with persons ages 1-6 years)

Score (%)	*Description*
100	Fully active, normal
90	Minor restrictions in physically strenuous activity
80	Active, but tires more quickly
70	Both, greater restrictions of, and less time spend in play activities
60	Up and around, but minimal active play, keeps busy with quieter activities
50	Gets dressed but lies around much of the day, no active play; able to participate in all quiet play activities
40	Mostly in bed; participates in quiet activities
30	In bed; needs assistance even for quiet play
20	Often sleeping; play entirely limited to very passive activities
10	Unresponsive
0	Dead

Appendix D

ABO INCOMPATIBILITY

Red Blood Cell - Incompatibility (Major):
Occasional patients may have antibodies directed against red blood cell antigens found on the donor's cells. These are generally ABO or Rh antigens, although incompatibility with other red cell antigens identified by donor-recipient crossmatch may occur. Although the volume of red blood cells (RBC) in most PBMC products will only be 2-5% of the product volume before infusion, the small quantity may cause a hemolytic transfusion reaction. According to the FHCRC policy it is generally acceptable to infuse a volume of about 10ml RBCs per product. If the recipient shows an anti-donor titer of ≥ 1:32 or the RBC volume is greater than 10ml (or >20ml in two products combined) the PBMC components should be RBC depleted by Starch Sedimentation (flowsheet below). *Refer to the Clinical Coordinator's Patient Information Sheet for instructions regarding management of a specific patient.*

Post transplant blood component support will be according to Standard Practice Guidelines.

Timing: Every attempt should be made to infuse red cell depleted PBMC products within 2 hours of depletion.

Expected Results: Red blood cell depleted PBMC products will contain < 10ml of red blood cells and ≥ 90% nucleated cell recovery.

Red Blood Cell - Incompatibility (Minor): Occasional donors may have antibodies directed against red blood cell antigens (ABO, Rh, or other antigen system) found on the recipient's cells. The risk of hemolysis of recipient red cells immediately after transplant is not of very much clinical import. Due to the high number of lymphocytes in the PBMC inoculum, recipients may be at much greater risk for a delayed type of hemolysis that can be severe. PBMC products contain < 200ml of plasma according to FHCRC policy and no deleterious effects have been observed so far. However, if donors show an anti-recipient titer ≥ 1:256, the PBMC component should be plasma depleted (see flowsheet below). *Refer to the Clinical Coordinator's Patient Information Sheet for instructions regarding management of a specific patient.*

Post transplant blood component support will be according to Standard Practice Guidelines.

Timing: Every attempt should be made to infuse plasma-depleted PBMC within 2 hours of depletion.

Expected Results: The plasma depletion should not affect the nucleated cell recovery.

Red Blood Cell – Bidirectional Incompatibility: Patients undergoing transplants for bidirectional RBC incompatibility should be managed according to both algorithms shown below. Most red cell depletion techniques also deplete plasma from the PBMC component with no additional cell loss. *Refer to the Clinical Coordinator's Patient Information Sheet for instructions regarding management of a specific patient.*

Post transplant blood component support will be according to Standard Practice Guidelines.

Appendix D (cont'd)

ABO INCOMPATIBILITY

MAJOR ABO INCOMPATIBLE			
Recipient anti-Donor titer	**≥ 1:32**	<20ml RBC total	• Infuse without modification
		>20ml RBC total	• RBC depletion of component
	≤ 1:16	•	Infuse without modification
MINOR ABO INCOMPATIBLE			
Donor anti-Recipient titer	**≥ 1:256**	Plasma depletion of component	
	≤ 1:128	Infuse without modification	

Appendix E

INFECTIOUS DISEASE GUIDELINES

Please note that the content of these PDFs is from the Fred Hutchinson Clinical Research Division Standard Practice Manual and does not contain research related procedures.

Herpes Simplex and Varicella Zoster Virus Prevention and Treatment

HSV-VZV.pdf

CMV Prevention: Surveillance and Preemptive Therapy

cmvprevention.pdf

CMV Disease: Diagnosis and Treatment

cmvdiseasetreatment.pdf

Antifungal Therapy Guidelines

antifungal_therapy.pdf

Pneumonia / Pneumocystis Carinii Prophylaxis

pneumocystisjiroveci.pdf

Antibiotic Prophylaxis for Encapsulated Bacteria in Allogeneic Patients with Chronic GvHD Requiring Immunosuppressive Therapy

Antibiotic.Prophylaxis-Encapsulated

Vaccinations

Vaccines.pdf

Foscarnet

foscarnet.pdf

Appendix F

GRADING OF ACUTE GRAFT-VERSUS-HOST DISEASE[a]

Severity of Individual Organ Involvement		
Skin	+1	a maculopapular eruption involving less than 25% of the body surface
	+2	a maculopapular eruption involving 25-50% of the body surface
	+3	generalized erythroderma
	+4	generalized erythroderma with bullous formation and often with desquamation
Liver	+1	bilirubin (2.0-3.0mg/100ml)
	+2	bilirubin (3-5.9mg/100ml)
	+3	bilirubin (6-14.9mg/100ml)
	+4	bilirubin > 15mg/100ml
Gut	Diarrhea is graded +1 to +4 in severity. Nausea and vomiting and/or anorexia caused by GVHD is assigned as +1 in severity. The severity of gut involvement is assigned to the most severe involvement noted. Patients with visible bloody diarrhea are at least stage +2 gut and grade +3 overall	
Diarrhea	+1	≤ 1000 ml of liquid stool/day* (≤ 15ml of stool/kg/day)†
	+2	>1,000 ml of stool/day* (> 15ml of stool/kg/day)†
	+3	>1,500 ml of stool/day* (> 20ml of stool/kg/day)†
	+4	2,000 ml of stool/day* (≥ 25ml of stool/kg/day)†

**In the absence of infectious/medical cause*
†For pediatric patients

Severity of GVHD	
Grade I	+1 to +2 skin rash
	No gut or liver involvement
Grade II	+1 to +3 skin rash
	+1 gastrointestinal involvement and/or +1 liver involvement
Grade III	+2 to +4 gastrointestinal involvement and/or
	+2 to +4 liver involvement with or without a rash
Grade IV	Pattern and severity of GVHD similar to grade 3 with extreme constitutional symptoms or death

a From “Graft-vs-host disease” Sullivan, Keith M. *Hematopoietic Cell Transplantation* Ed: D. Thomas, K. Blume, S. Forman, Blackwell Sciences; 1999, pages 518-519.

Appendix G

CHRONIC GRAFT-VERSUS-HOST DISEASE (GVHD)

Chronic GVHD in allogeneic transplant recipients resembles autoimmune disorders such as scleroderma, Sjogren syndrome, primary biliary cirrhosis, lichen planus, wasting syndrome, bronchiolitis obliterans among others manifestations (see below). Approximately 50% of patients will develop this complication within 6 months after the transplant despite continued treatment with immunosuppressive medications. Close monitoring is recommended during the first 2 years after allogeneic stem cell transplantation so that appropriate treatment can be instituted promptly in patients who develop chronic GVHD. Debilitation, joint contractures and profound immunosuppression resulting in recurrent bacterial infections are prominent characteristics of untreated chronic GVHD.

A. Classification of Chronic GVHD

The purpose of this classification is to identify patients with cGVHD who need long-term systemic immunosuppression according to clinical and laboratory findings and risk factors at the time of initial diagnosis. In addition, a morbidity scale has been developed to help grade the severity of manifestation of chronic GVHD (Appendix D) at the time of diagnosis, when changes in treatment are made and when assessing treatment response.

1. **Chronic GVHD not requiring systemic treatment: mild abnormalities involving a single site, with platelet count >100,000 and no steroid treatment at the onset of chronic GVHD**
   a) Oral abnormalities consistent with cGVHD, a positive skin or lip biopsy, and no other manifestations of cGVHD.
   b) Mild liver test abnormalities (alkaline phosphatase ≤2 x upper limit of normal, AST or ALT ≤3 x upper limit of normal and total bilirubin ≤1.6) with positive skin or lip biopsy, and no other manifestations of cGVHD.
   c) Less than 6 papulosquamous plaques, macular-papular or lichenoid rash involving <20% of body surface area (BSA), dyspigmentation involving <20% BSA, or erythema involving <50% BSA, positive skin biopsy, and no other manifestations of cGVHD.
   d) Ocular sicca (Schirmer's test ≤5mm with no more than minimal ocular symptoms), positive skin or lip biopsy, and no other manifestations of cGVHD.
   e) Vaginal or vulvar abnormalities with positive biopsy, and no other manifestations of cGVHD.
2. **Chronic GVHD requiring systemic treatment: more severe abnormalities or involvement of multiple sites, or platelet count <100,000, or steroid treatment at the onset of chronic GVHD**
   a) Involvement of two or more organs with symptoms or signs of cGVHD, with biopsy documentation of cGVHD in any organ.
   b) ≥15% base line body weight loss not due to other causes, with biopsy documentation of cGVHD in any organ.
   c) Skin involvement more extensive than defined for clinical limited cGVHD, confirmed by biopsy.
   d) Scleroderma or morphea.
   e) Onycholysis or onychodystrophy thought to represent cGVHD, with documentation of cGVHD in any organ.
   f) Decreased range of motion in wrist or ankle extension due to fasciitis caused by cGVHD.
   g) Contractures thought to represent cGVHD.
   h) Oral involvement with functional impairment, refractory to topical treatment.
   i) Vaginal involvement with functional impairment, refractory to topical treatment.
   j) Bronchiolitis obliterans not due to other causes.
   k) Positive liver biopsy; or abnormal liver function tests not due to other causes with alkaline phosphatase >2 x upper limit of normal, AST or ALT >3 x upper limit of normal, or total bilirubin >1.6, and documentation of cGVHD in any organ.
   l) Positive upper or lower GI biopsy.
   m) Fasciitis or serositis thought to represent cGVHD and not due to other causes

B. Physical manifestations of Chronic GVHD

Manifestations that are distinctive for chronic GVHD can begin before day +100 after the transplant, and manifestations that are typical of acute GVHD can persist long after day +100. For this reason, the differential diagnosis between acute and chronic GVHD cannot be made solely according to the time interval from transplant. The diagnosis of chronic GVHD requires at least one manifestation that is distinctive for chronic GVHD (*identified by italic print below*) as opposed to acute GVHD. In all cases, infection and others causes must be
ruled out in the differential diagnosis of chronic GVHD.

Karnofsky or Lansky Clinical Performance scores <60%, ≥15% weight loss, and recurrent infections are usually signs of clinical extensive chronic GVHD. Abnormalities that could indicate chronic GVHD are categorized by organ system are listed below (*italic print identifies manifestation more distinct of chronic GVHD):*

Skin	Erythema, dryness, pruritis, macular-papular or urticarial rash, *pigmentary changes (i.e., hyperpigmentation, vitiligo), mottling, papulosquamous or lichenoid plaques, hyperkeratosis, exfoliation (ichthyosis), nodules, scleroderma, morphea (one or several circumscribed, indurated and shiny lesions)*. The extent of skin involvement and the skin thickness score for patients with scleroderma needs to be recorded at the time of diagnosis, when changes in treatment are made and when assessing treatment response. Medical photos are also useful for assessing the extent of skin involvement and response to treatment.
Nails	*A. Ridging, onychodystrophy, onycholysis*
Hair	*Premature graying (scalp hair, eyelashes, eyebrows), thinning scalp hair, alopecia, decreased body hair*
Mouth	Dryness, burning, gingivitis, mucositis, striae, *dryness, atrophy, erythema, lichenoid changes, ulcers, labial atrophy or pigmentary changes, tightness around the mouth, sensitivity to acidic, strong flavors, heat or cold, tooth decay*
Eyes	*Dryness, burning, blurring, gritty eyes, photophobia, pain*
Vagina/vulva	*Dryness, dyspareunia, stricture or stenosis, erythema, atrophy or lichenoid changes not induced by ovarian failure or other causes*
Liver	Jaundice and elevated liver function tests not due to other causes (see laboratory tests)
Lung	*Bronchiolitis obliterans (see diagnostic indicators), cough, wheezing, dyspnea on exertion, history of recurrent bronchitis or sinusitis*
GI	Anorexia, nausea, vomiting, diarrhea, *malabsorption, dysphagia, odynophagia*
Myofascial	*Stiffness and tightness with restriction of movement, occasionally with swelling, pain, cramping, erythema and induration, most commonly affecting the forearms, wrists and hands, ankles, legs and feet, inability to extend the wrists without flexing the fingers or the elbows, contractures*
Muscle	*B. Proximal muscle weakness, cramping*
Skeletal	*Arthralgia of large proximal girdle joints and sometimes smaller joints*
Serosal	*Unexplained effusions involving the pleural, pericardial, or peritoneal cavities not due to venocclusive disease of the liver, cardiac insufficiency, malignancy, infection, GM-CSF toxicity or other causes*

C. Laboratory Testing and Diagnostic Indicators of Chronic GVHD

Eye	*Schirmer's test with a mean value ≤ 5 mm at 5 minutes, or values of 6-10 mm in patients who have sicca symptoms, or keratitis detected by slit lamp examination*
Liver	Elevated liver function tests not due to other causes (alkaline phosphatase ≥2 x upper limit, of normal, AST or ALT >3 x upper limit of normal or total serum bilirubin ≥1.6)
Lung	*New obstructive lung defect defined as an FEV1 <80% of predicted with either an FEF 25-75 <65% of predicted or RV >120% of predicted, or a decrease of FEV1/FVC by >12% within a period of less than 1 year, though not to be caused by an infectious process, asthma or recurrent aspiration from the sinuses or from gastroesophageal reflux. In the absence of GVHD in any other organ, the diagnosis of bronchiolitis obliterans requires negative microbiological tests from bronchoalveolar lavage, evidence of air trapping by high resolution end-expiratory and end-inspiratory CAT scan of the lungs, or confirmation by thoracoscopic biopsy.*
Esophagus	*Esophageal web formation, stricture or dysmotility demonstrated by barium swallow, endoscopy or manometry*
Intestine	Endoscopic findings of mucosal edema and erythema or focal erosions with histological changes of apoptotic epithelial cells and crypt cell drop out. Patients with unresolved acute GVHD may have more severe intestinal mucosal lesions including ulcers and mucosal sloughing.
Muscle	*Elevated CPK or aldolase, EMG findings consistent with myositis with biopsy revealing no other etiological process*
Blood	Thrombocytopenia (usually 20,000-100,000/□l), eosinophilia (> 0.4 x 10^3/uL), hypogammaglobulinemia. Hypergammaglobulinemia and autoantibodies occur in some cases.

D. Guidelines for Treatment of Chronic GVHD after allogeneic HSCT

We strongly recommend that you consult the LTFU office before beginning treatment for chronic GVHD and before making changes in immunosuppressive treatment. Clinical trials should always be considered because current standard therapies are associated with high morbidity and decreased survival for patients with high risk chronic GVHD

Standard treatment of chronic GVHD usually begins with administration of glucocorticoids (1mg/kg/day) followed by taper to eventually reach an alternate-day regimen, with or without daily cyclosporine or tacrolimus (FK506). Other medications used for treatment of corticosteroid-resistant chronic GVHD are summarized on the next page. Telephone consultation with the LTFU medical team is available to you, seven days a week, to discuss appropriate treatment and provide other follow up recommendations. In addition to immunosuppressive treatment, antibiotic prophylaxis for encapsulated bacterial infections and PCP must be given to all patients being treated for chronic GVHD (see Appendix E).

The duration of systemic immunosuppressive treatment of chronic GVHD varies but requires at least one year of therapy. Approximately 80% of patients require systemic immunosuppressive for 2 years and 40% of them requires therapy for at least 4 years.

Adapted From: Long-Term Follow-up After Hematopoietic Stem Cell Transplant General Guidelines For Referring Physicians, Fred Hutchinson Cancer Research Center Standard Practice Manual, Section X, Chronic Graft Versus Host Disease (GVHD), **Nov/2003 Version**

Appendix H
EVALUATION OF DISEASE RESPONSE

General guidelines

(A) Any assessment of response must include a bone marrow aspirate. Patients with myelofibrosis will also require a biopsy.
(B) All bone marrow aspirates must also have cytogenetics analysis. For some patients, there may be additional disease-specific FISH or PCR-assessed molecular markers that can be used to determine response.
(C) Regimen-related toxicity, for example due to drug adverse effect, severe infection, or GVHD must be excluded as causes of peripheral blood cytopenias.

Definition of Response

1 Definition of Response : MDS/CMML

(A) *Complete Response*

- Bone marrow rating: Normal maturation of all cell lines, without morphologic dysplasia and with< 5% myeloblast.
- Peripheral blood rating: no peripheral blasts and no dysplasia.

(B) *Complete Response with normal blood counts*[33]

- Bone marrow rating: Normal maturation of all cell lines, without morphologic dysplasia and < 5% myeloblasts.
- Peripheral blood rating: Hb >11 g/dL, neutrophils $\geq$1500/mm^3, platelets >100 000/mm^3, blast 0%, no dysplasia.

(C) *Progressive Disease*

- Any evidence by morphologic or flow cytometric evaluation of the bone marrow aspirate of new blasts (>5%).

2 Definition of Response: Agnogenic Myeloid Metaplasia[34]

(A) *Complete Response*

In patients with marrow fibrosis:

- Achievement of $\geq$95% donor chimerism in the bone marrow, and:
- evidence of regression of fibrosis as determined by sequential bone marrow biopsies (however residual fibrosis may be present).

In patients with myelodysplastic features or with leukemic transformation:

- achievement of $\geq$95% donor chimerism in the marrow, and:
- regression of marrow fibrosis, and:
- absence of leukemic blasts, and:
- disappearance of dysplastic changes.

(B) *Progressive Disease*

- Any evidence of blastic transformation.

3 Definition of Response: Atypical CML

(A) *Complete Response*

- normal peripheral blood counts and leukocyte differential OR achievement of ≥95% donor chimerism in the bone marrow, and:
- resolution of pretreatment cytogenetic abnormality, and:
- Normal maturation of all cell lines, without morphologic dysplasia (if present pre-treatment), and:
- < 5% myeloblasts.

(B) *Progressive Disease*

- Any evidence of blastic transformation.

4 Definition of Response: Polycythemia Vera and Essential Thrombocythemia

(A) *Complete Response*

- Hematocrit ≤ 45% in the absence of phlebotomy, normal platelet count (< 400,000/ml) or achievement of ≥95% donor chimerism in the bone marrow.

(B) *Progressive Disease*

- Erythrocytosis, thrombocytosis, or evidence of leukemic transformation.

5 Definition of Response: PNH

(A) *Complete Response*

- Greater than 95% of the red blood cells not expressing the anchor proteins (documented by flow cytometry) in the absence of transfusions or achievement of ≥95% donor chimerism in the bone marrow.
- No clinical evidence of hemolysis related to PNH

Appendix I
STUDY COORDINATOR'S MANUAL

I. Introduction
The mixed chimerism protocols have been opened to multiple sites to increase the referral base and accrual. Because of this expansion of collaborators, the data collection procedures are being revised. The procedure manual was created to assure consistency of data reporting across the centers and to assure compliance with regulations. General expectations of collaborators are that they will comply with appropriate regulatory requirements, specified protocol requirements, and provide outcome data.

The manual translates working procedures for study coordination. Its goal is to describe the procedures with sufficient clarity to ensure that all study centers will use the same procedures and follow-up schedules for participant data management and reporting. Changes to the manual and relevant forms will be made as soon as practical and will become effective on receipt of the revised procedures at the study centers, unless otherwise noticed.

II. Institutional Review Board Review of Protocols and Modifications
All research protocols proposed for use that involves human subjects must be reviewed and approved by the Institutional Review Board (IRB) prior to implementation. New protocols will undergo review at the FHCRC IRB and then will be distributed to sites that wish to participate for their IRB's review. For Centers that have a Federal Wide Assurance (FWA), formal collaboration includes submission of a form 310 and a copy of the IRB approved protocol and consent forms to the FHCRC. For sites without a FWA, an FWA form needs to be filed. Once the paperwork is submitted to the Office for Human Research Protection, the approval process can take up to a couple of months, and must be completed before collaboration on a protocol can begin.

In addition, all amendments and/or revisions to on-going, approved activities must be submitted for review and approved prior to implementation at an institution. No revisions may be implemented at outside institutions without the prior approval of the FHCRC Principal Investigator. The FHCRC and the local site's IRB must review all protocol activities at least once annually. This must be done within 365 days of the last review regardless of the policies of the institution. A copy of annual renewal approvals must be received for collaboration to continue for the next year.

III. Registrations
Collaborating Institutions: The principal investigator of the collaborating institution who will register the patient with the FHCRC will identify eligible patients. Registration will include completion of the eligibility checklist/demographic form. This form will be faxed (206-667-5378) prior to treatment initiation. Patients should be registered prior to treatment initiation for valid registration

IV. Reporting Adverse Events
The following guidelines are the minimum serious adverse event (SAE) reporting guidelines for Category 1 and 2 studies conducted at the Fred Hutchinson Cancer Research Center.

Expedited Reporting Requirements
All unexpected and **serious adverse events which may be due to study treatment or intervention** must **be reported to the FHCRC Institutional Review Office as soon as possible but within at least 10 calendar days of the investigator learning of the event.**

Appendix I (cont'd)
STUDY COORDINATOR'S MANUAL

Definitions
Adverse Event - Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product, medical treatment or procedure and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, medical treatment or procedure whether or not considered related to the medicinal product.

Life-threatening Adverse Event – Any adverse event that places the patient or subject, in view of the investigator, at immediate risk of death from the reaction. Study toxicities are graded using the adapted NCI Common Toxicity Criteria (where appropriate use the criteria for transplant patients.) All Grade 4 (life-threatening) toxicities occurring between start of conditioning to day 200 that meet expedited reporting requirements must be reported as soon as possible but within at least 10 calendar days of the investigator learning of the event.

Unexpected Adverse Event – An adverse event, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved investigational product or package insert/summary of product characteristics for an approved product). If applicable product information is not available, such as for studies that do not involve pharmaceutical products or devices, an unexpected adverse event is an adverse event that was not described in the study protocol or informed consent.

- **Serious Adverse Event (SAE)** – Any adverse event occurring that results in any of the following outcomes:
- Death – start of conditioning to day 200, regardless of cause.
- life-threatening adverse event (see above).
- persistent or significant disability/incapacity.
- congenital anomaly.
- requires intervention to prevent permanent impairment or damage.

Hospitalization, in general, will not be considered a serious adverse event as approximately half of evaluable MRD patients AND the majority of evaluable URD patients receiving nonmyeloablative transplants were hospitalized. Hospitalization will be considered a serious adverse event if it fulfills the criteria for a serious and unexpected adverse event as described above.

To ensure no confusion or misunderstanding exist of the differences between the terms "serious" and "severe," which are not synonymous the following note of clarification is provided:

The term "severe" is often used to describe the intensity (severity) or a specific event (as in mild, moderate or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as "serious," which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a patient's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory obligations.

Appendix I (cont'd)
STUDY COORDINATOR'S MANUAL

Attribution - The FHCRC designation for the determination of whether an adverse event is related to a medical product, treatment or procedure will be as follows:

- Related – includes adverse events that are definitely, probably, or possibly related to the medical treatment or procedure.
- Not Related – includes adverse events are doubtfully related or clearly not related to the medical treatment or procedure.

The FHCRC Serious Adverse Event (SAE) Report Form should be completed for all adverse events that meet the expedited reporting requirements. All available information should be submitted but it is acceptable to fax an incomplete report form at the initial report. A completed report should be faxed as soon as possible but must be received within 10 calendar days.

It is the responsibility of the FHCRC Principal Investigator to notify the sponsor, NIH, FDA or other agencies of serious adverse events as required in the protocol.

Serious adverse events that do not meet the requirement for expedited reporting (not related to study treatment or expected) will be reported to the IRB as part of the annual renewal of the protocol.

FHCRC is acting as the Coordinating Center for this multi-institutional study, and it is the responsibility of the FHCRC Principal Investigator (or designee) to complete the FHCRC Serious Adverse Event Report for all serious adverse events that meet the expedited reporting requirements that are received from the participating sites.

Procedure for Reporting Serious and Unexpected Adverse Events from Participating Sites
Regulations defining the responsibilities for reporting serious and unexpected adverse reactions are defined above. Serious and unexpected adverse events must be reported to the FHCRC Investigator within 10 days of learning of the event. This includes patient deaths (serious, unexpected, and related/possibly related), regardless of cause, occurring start of conditioning to day 200 post-transplant procedure. The immediate telephone report must be followed by faxed comments to the FHCRC Trial Coordinator at **(206) 667-5378.** This will be followed by detailed written report (See **Appendix J**) within 10 working days. The report must include the date and time of onset, severity and duration of the event, the relationship to the study, the treatment given and eventual outcome. Follow-up information to a SAE report must be submitted as soon as the relevant information is available.

Obligation of Investigators
All grade 3 or 4 adverse events (or highly unusual grade 2 adverse events), which occur between start of conditioning to day 100 during the study will be recorded on the Case Report Form (**Appendix M)**. These adverse events which are observed by the Investigator or reported by the patient, whether or not attributed to the study, will be reported on the Case Report Form using the modified (for HSCT) NCI Common Toxicity Criteria (**Appendix P**). Attributes will include a description, date of onset, maximum severity, and assessment of relationship to the study agent or other suspect agent(s).

Adverse events will be graded accordingly: 0 = none, 1 = mild, 2 = moderate, 3 = severe, 4 = life threatening or debilitating, and 5 = fatal. All Grade 4 (life-threatening) or Grade 5 (fatal) events on the Adapted HSCT NCI scale meet expedited reporting requirements.

Appendix I (cont'd)
STUDY COORDINATOR'S MANUAL

Association or relatedness to the study agent will be graded as follows: 1 = unrelated, 2 = unlikely, 3 = possibly, 4 = probably, and 5 = definitely related.

V. Case Report Forms

Case report forms must be completed for all patients registered onto the protocols and submitted to the FHCRC data coordinating center. The first case report form (day 28) is due on day 50. For outside centers a Staging Form must accompany the form with the patient staging at registration, day 28, day 56, day 84 and day 100. Staging forms should also be completed with each Follow Up Form completed on day 180, 1 year, 1.5 years, 2 years, 3 years, and yearly thereafter. For Outside Centers, case report forms are expected to be submitted no later than 30 days following the scheduled follow up date.

VI. Protocol Monitoring

As the coordinating center, FHCRC will monitor accrual at the outside institutions. The guidelines below are intended to guide the reviewers in their assessment of items that significantly alter the clinical effectiveness of the treatment or the evaluation of its toxicity.

A. Registration/Randomization
   1. Patient was registered prior to treatment and approval by FHCRC PI occurs prior to randomization.
   2. Information given at registration represents actual data in medical records (stage, diagnosis, cell type, etc.)

B. Informed Consent/IRB Approval Dates
   1. The consent was signed prior to registration
   2. The consent is in language was approved by the institution's IRB. IRB approval and reapproval are documented including appropriate use of full-board review and proper review of appropriate amendments or revisions
   3. Consent was dated and has written witness signature. IRB approval was obtained prior to the patient signing the consent form and start of treatment.

C. Patient Eligibility
   1. Eligibility criteria and exclusion criteria were met
   2. Treatment/Intervention Administration
   3. Doses were modified according to protocol
   4. Accurate documentation of drug administration

D. Study Tests/Evaluation
   1. Protocol specified laboratory tests or diagnostic studies are available
   2. Appropriate record of protocol intervention is documented.

E. Study Events/Adverse Drug Experience
   1. Serious Adverse Evens reported according to protocol specifications

F. Follow-Up
   1. Disease status assessed according to the required protocol guidelines documenting response to treatment.
   2. Accurate determination of cancer progression

Appendix J

Fred Hutchinson Cancer Research Center/ Clinical Research Division / Institutional Review Office

SERIOUS ADVERSE EVENT REPORT (SAE) Form IRO-08

FHCRC IR File Number: _____ FHCRC Protocol Number: _____

FHCRC Unique Patient #: _____ ☐ FHCRC/SCCA ☐ Other

Gender: ☐ Male ☐ Female Age: _____

FHCRC Principal Investigator: _____

Phone Number: _____ Mailstop: _____

Date of Report: _____/_____/_____

☐ Initial Report _____ ☐ Follow-Up Report #: _____ ☐ Other

Date Study Staff Became Aware of Event: _____

Date Serious Adverse Event Began: _____/_____/_____

Date Ended: _____/_____/_____ Or ☐ Ongoing *(if ongoing – must submit follow up report)*

Adverse Event: _____

_____

Describe the Serious Adverse Event including a summary of all relevant clinical information. (Or attach a MedWatch Form or other SAE reporting form if one has been completed.) Use Page 2, if necessary:_____

Outcomes Attributed to adverse event: *(Check all that apply)*

☐ Death: _____/_____/_____
☐ Life-Threatening
☐ Hospitalization (initial or prolonged)
☐ Disability
☐ Congenital Anomaly
☐ Required intervention to prevent permanent impairment/damage

Specify Agent(s) and/or Procedure(s) involved in this protocol:

#1: _____
Pharmaceutical product/medical treatment/procedure
☐ Not Related (Unrelated, Unlikely)
☐ Related (Possible, Probable, Definite)

#2: _____
Pharmaceutical product/medical treatment/procedure
☐ Not Related (Unrelated, Unlikely)
☐ Related (Possible, Probable, Definite)

☐ Follow-up Report Required ☐ Final Report (PI must sign final report)

Report Completed by: Date:

_____ _____

The PI has determined that the consent form must be revised: ☐ Yes ☐ No

Does this study involve the deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA, into human subjects (human gene transfer)? ☐ Yes ☐ No If yes and the activity involves the SCCA outpatient clinic, a copy of this Protocol Modification Form and any supporting documents to be reviewed and approved, will be forwarded to the FHCRC's Institutional Biosafety Committee (IBC) by the Protocol Office (Mailstop: LM-230).

_____ _____

Signature of Principal Investigator Date:

Appendix J (cont'd)

Fred Hutchinson Cancer Research Center / Clinical Research Division / Institutional Review Office

SERIOUS ADVERSE EVENT REPORT (SAE) Form IRO-08

page 2

FHCRC IR File Number: _____ FHCRC Protocol Number: _____

FHCRC Unique Patient #: _____ Date of Report: _____

Describe the Serious Adverse Event including a summary of all relevant clinical information.

Appendix K

NOTICE OF DEATH

Patient ID: _____ Date of Death: _____

Place of Event: _____

Apparent cause of death *(Please be specific. Attach hospital summary or death summary when possible):*

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

Form completed by: _____ Date: _____

Appendix L
PROTOCOL 2056.00
PATIENT DEMOGRAPHICS AND ELIGIBILITY FORM

Please fax this completed form to (206) 667-5378 for patient registration.
Questions regarding eligibility should be directed to the Brenda Sandmaier, MD (206-667-4961).

UPN#: _____

Patient Name: _____ _____ _____
(Last) *(First)* *(MI)*

Date of Birth: _____ / _____ / _____ Age:_____
(Mo) (Day) (Year)

Gender *(choose one):*
☐ Male ☐ Female ☐ Unknown

Patient Diagnosis:_____

Planned Day 0: _____/_____/_____
(Mo) (Day) (Year)

Status at Transplant:_____

Ethnicity *(choose one): Instruct the research subject to select one of the following.*

☐ **Hispanic** *(A person of Cuban, Mexican, Puerto Rican, South or Central American, or other Spanish culture or origin, regardless of race. Term "Spanish Origin" can also be used in addition to "Hispanic" or "Latino".*
☐ **Not Hispanic or Latino**
☐ Declined to report

Race *(check all that apply): Instruct the research subject to select one or more of the following.*

☐ **American Indian/Alaska Native** *(A person having origins in any of the original peoples of North, Central, or South America, and who maintains tribal affiliations or community attachment).*
☐ **Asian** *(A person having origins in any of the original peoples of the Far East, Southeast, Asia, or the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand and Vietnam).*
☐ **Native Hawaiian/Pacific Islander** *(A person having origins in any of the original peoples of Hawaii, Guam, Samoa or other Pacific Islands).*
☐ **Black/African American** *(A person having origins in any of the black racial groups of Africa).*
☐ **White** *(A person having origins in any of the original peoples of Europe, the Middle East or North Africa).*
☐ Research subject does not know race
☐ Declined to report

PROTOCOL ELIGIBILITY

Inclusion Criteria

1. Yes ☐ No ☐ Patient has signed IRB approved consent form.
   Date: _____
   IRB File Number: _____
   Date of IRB Approval: _____

2. Yes ☐ No ☐ Called FHCRC for Arm and Dose confirmation. Date. _____

☐ **Arm A: MPD or MDS-RA/RARS, or PNH**

TBI DOSE LEVEL: ☐ 1st dose level: 300 cGy
☐ 2nd dose level: 400 cGy
☐ 3rd dose level: 450 cGy

OR

☐ **Arm B: MDS-RAEB or CMML**

TBI DOSE LEVEL: ☐ 1st dose level: 300 cGy
☐ 2nd dose level: 400 cGy
☐ 3rd dose level: 450 cGy

One of the following criteria questions (3-4) must be marked "Yes" for the patient to enroll in this study

3. Yes ☐ No ☐ Related donor who is genotypically or phenotypically HLA-identical.

4. Yes ☐ No ☐ Unrelated donors who are prospectively:
   a. Matched for HLA-A,B,C, DRB1 and DQB1 alleles by high resolution typing
      AND
   b. Only a single allele disparity will be allowed for HLA-A, B, or C as defined by high resolution typing (See appendix O for other donor selection details)

Patient					
A: _____	A:_____	C:_____	C:_____	B:_____	B:_____
DRB1:_____	DRB1:_____	DQB1: _____	DQB1: _____		
Donor					
A:_____	A:_____	C:_____	C:_____	B:_____	B:_____
DRB1:_____	DRB1:_____	DQB1: _____	DQB1: _____		

c. Yes ☐ No ☐ **NA** ☐ Have a negative anti-donor cytotoxic crossmatch. Cytotoxic crossmatch **not done as patient and donor are phenotypically identical by molecular methods.**

d. Yes ☐ No ☐ Patient and donor pairs must not be homozygous at mismatched allele.

---

*One of the following criteria questions (5-6) must be marked "**Yes**" for the patient to enroll in this study.*

5. Yes ☐ No ☐ Age ≥ 50 years and <75 years with CMML, or previously untreated MDS or MPD.

6. Yes ☐ No ☐ Age < 50 years of age at high risk for regimen related toxicity using standard high dose regimens. Factors considered high risk include pre-existing conditions such as a chronic disease affecting kidneys, liver, lungs, or heart or previous failed HCT. All children < 12 years must be discussed with the FHCRC PI *(Brenda Sandmaier, MD 206-667-4961)* prior to registration.

   Pre-existing condition(s) precluding conventional tx: _____
   ***Patients ≤ 50 years of age who have received previous autologous transplantation do not require patient review committee approval. All children < 12 years must be discussed with the FHCRC PI (Brenda Sandmaier, MD 206-667-4961) prior to registration.*

---

*One of the following criteria questions (7 - 14) must be marked "**Yes**" for the patient to enroll in this study.*

7. Yes ☐ No ☐ MDS classifiable by the WHO system (see appendix R) as RA, RARS, refractory cytopenia with multilineage dysplasia (RCMD), RCMD and ringed sideroblasts (RCMD-RS) or RAEB. No previous myelosuppressive therapy. For the purpose of this protocol myelosuppressive chemotherapy will be

defined as chemotherapy given with the intent of inducing a complete remission (e.g. standard 7+3, HIDAC, or mylotarg). Patients must have < 10% marrow blasts. Fewer than 10% marrow blasts must be documented by marrow examination within 3 weeks of initiation of conditioning.

8. Yes ☐ No ☐ Philadelphia chromosome-negative patients with a diagnosis of atypical CML. No previous myelosuppressive therapy. For the purpose of this protocol myelosuppressive chemotherapy will be defined as chemotherapy given with the intent of inducing a complete remission (e.g. standard 7+3, HIDAC, or mylotarg). Must have < 10% blasts at HCT.*

9. Yes ☐ No ☐ Polycythemia vera with persistent thrombotic or hemorrhagic complications despite conventional therapy, or who have progressed to postpolycythemic marrow fibrosis. No previous myelosuppressive therapy. For the purpose of this protocol myelosuppressive chemotherapy will be defined as chemotherapy given with the intent of inducing a complete remission (e.g. standard 7+3, HIDAC, or mylotarg). Must have < 10% blasts at HCT.*

10. Yes ☐ No ☐ Chronic idiopathic myelofibrosis with peripheral blood cytopenias. No previous myelosuppressive therapy. Must have < 10% blasts at HCT.*

11. Yes ☐ No ☐ Essential thrombocythemia with persistent thrombotic or hemorrhagic complications despite conventional therapy, or who have progressed to myelofibrosis. No previous myelosuppressive therapy. Must have < 10% blasts at HCT.*

12. Yes ☐ No ☐ Agnogenic myeloid metaplasia with peripheral blood cytopenias. No previous myelosuppressive therapy. Must have < 10% blasts at HCT.*

13. Yes ☐ No ☐ <u>Chronic Myelomonocytic leukemia</u>. Patients with CMML1 who have not received myelosuppressive therapy must have < 10% blasts at HCT.* Patients with CMML who have progressed beyond CMML1 and have received myelosuppressive chemotherapy must have <5% marrow blasts. *

14. Yes ☐ No ☐ Paroxysmal nocturnal hemoglobinuria. Patients with the non-aplastic form of PNH (cellular bone marrow) who have had a history of life-threatening complications of their disease including thrombotic events, severe hemolysis or Budd Chiari syndrome are eligible. Other patients may be considered following approval at PCC and approval by the protocol Principal investigator.

*<u>**Note**: Date of most recent marrow examination (must be within 3 weeks of initiation of conditioning regimen). _____/__ /_____</u>

Exclusion Criteria

*Each of the following questions must be marked "**No" or "NA**" for the patient to enroll in this study.*

15. Yes ☐ No ☐ Bone marrow documenting blast count ≥10% or ≥ 5% in CMML patients who have progressed beyond CMML1 and received myelosuppressive chemotherapy.

16. Yes ☐ No ☐ Active CNS involvement of disease (if LP requirement, see **Appendix N)**

17. Yes ☐ No ☐ Presence of ≥5% circulating leukemic blasts (in the peripheral blood) detected by standard pathology.

18. Yes ☐ No ☐ Patients with active non-hematologic malignancies (except non- melanoma skin cancers).
This exclusion does not apply to patients with non-hematologic malignancies that do not require therapy

19. Yes ☐ No ☐ Patients with a history of non-hematologic malignancies (except non-melanoma skin cancers) currently in a complete remission, who are less than 5 years from the time of complete remission, and have a >20% risk of disease recurrence.

20. Yes ☐ No ☐ NA ☐ Fertile men or women unwilling to use contraceptive techniques during and for 12 months following treatment.

21. Yes ☐ No ☐ NA ☐ Females who are pregnant or breastfeeding.

22. Yes ☐ No ☐ Fungal infections with radiological progression after receipt of amphotericin B or active triazole for > 1 month.

23. Organ Dysfunction

Please check yes if patient meets any of the following.

Yes ☐ No ☐ Cardiac: Symptomatic coronary artery disease or ejection fraction < 35% (or, if unable to obtain ejection fraction, shortening fraction of <26%). Ejection fraction is required if age > 50 years or there is a history of anthracycline exposure or history of cardiac disease.

NOTE: If shortening fraction is <26%, a cardiology consult is required. The PI of the study must approve eligibility

PI Signature: _____ Date: _____

Yes ☐ No ☐ Pulmonary: DLCO < 35%, TLC <35%, FEV1 <35% and/or receiving supplementary continuous oxygen.

Additionally, the FHCRC PI of the study must approve of enrollment of all patients with pulmonary nodules.

PI Signature: _____ Date: _____

Yes ☐ No ☐ Liver function abnormalities: Patient with clinical or laboratory evidence of liver disease will be evaluated for the cause of liver disease, its clinical severity in terms of liver function, bridging fibrosis, and the degree of portal hypertension. The patient will be excluded if he/she is found to have fulminant liver failure, cirrhosis of the liver with evidence of portal hypertension, alcoholic hepatitis, esophageal varices, a history of bleeding esophageal varices, hepatic encephalopathy, uncorrectable hepatic synthetic dysfunction evinced by prolongation of the prothrombin time, ascites related to portal hypertension, bacterial or fungal liver abscess, biliary obstruction, chronic viral hepatitis with total serum bilirubin >3mg/dL, or symptomatic biliary disease.

24. Yes ☐ No ☐ Karnofsky Performance Score < 70 (adult patients) or Lansky-Play Performance score < 70 (pediatric patients).

25. Yes ☐ No ☐ Patient is HIV-positive.

26. Yes ☐ No ☐ Life expectancy severely limited by diseases other than malignancy.

27. Yes ☐ No ☐ Severe psychological illness such as major psychosis (e.g. schizophrenia), major bipolar depression, or suicidal situational depression.

28. Yes ☐ No ☐ Patient has an active bacterial infection.

29. Yes ☐ No ☐ Patient has received chemotherapy (with the exception of hydroxyurea and anagrelide) within 21 days of initiation of conditioning.

***Note – the HCT-Comorbidity score is: _____ (fax HCT-CI worksheet with registration—see Appendix Q)**

Signature of person completing form: _____ Date: _____

Signature of Principal Investigator: _____ Date: _____

Appendix M

CORE CASE REPORT FORMS

Acrobat Document

Appendix N

INTRATHECAL THERAPY ADMINISTRATION

Please note that the content of this PDF is from the Fred Hutchinson Clinical Research Division Standard Practice Manual and does not contain research related procedures.

intrathecaltherapy-combined.pdf

Appendix O

HLA MATCHING REQUIREMENTS FOR UNRELATED DONORS AT THE SCCA / FRED HUTCHINSON ALLIED SYSTEM

Human Leukocyte Antigen (HLA) Terminology. The HLA region consists of genes that encode two classes of HLA molecules. **HLA class I molecules**, HLA-A, -B, and –C, are composed of a single glycoprotein chain that is expressed in association with □2-microglobulin on most tissue cells. **HLA class II molecules**, HLA-DR, -DQ, and –DP, are heterodimers consisting of □□and □□glycoprotein chains. HLA class I and HLA class II molecules are highly polymorphic.

HLA Typing Methods. At the Seattle Cancer Care Alliance Clinical Immunogenetics Laboratory (CIL) DNA-based methods of HLA-A, B, C, DRB1, DQB1 typing are now performed routinely. **High resolution** typing is required to define individual alleles and the level of mismatching between donor and recipient. **High resolution** data are reported with four or more digits (e.g., A*0201, A*0205, B*1504, or DRB1*0401**). A current listing of recognized HLA alleles and their sequences can be found at the Immunogenetics/HLA sequence database website at** www.anthonynolan.org.uk/HIG/data.html**.**

Initial typing reports obtained through the international marrow donor registries may consist of **intermediate resolution** typing. **Intermediate resolution** defines alleles in groups of related families historically defined as ***antigens*** by alloantisera. **Intermediate resolution** typing results are reported as two digits (e.g., A*02, B*15, or DRB1*04). In cases where the HLA-A, B and C loci are typed at intermediate resolution and high resolution data are not available, it should be understood that unidentified allele disparity might be present.

Donor Selection. Final selection of an unrelated donor should be based upon results of **high resolution** typing of HLA-A, B, C, DRB1, DQB1 alleles. Cross match assay is not required when high resolution typing indicates matching for HLA-A, B, C, DRB1 and DQB1 AND the platelet reactive antibody (PRA) screen is not elevated (defined as ≤10%). A negative cross match test result is required for final donor selection in the following situations: 1) PRA screen is positive (>10%), or 2) high resolution typing indicates mismatching for one or more HLA-A, B, C, DRB1 and DQB1 alleles. A positive anti-donor cytotoxic crossmatch absolutely excludes the donor.

Donor Selection Criteria. Protocols and treatment plans must specify donor inclusion and exclusion criteria, using terminology indicated below.

Donor inclusion criteria **must specify** 1) the allowable genetic relationship between the patient and donor (related and/or unrelated), 2) the allowable limits of mismatch, and if applicable 3) any modification of mismatch criteria according to type of disease or patient characteristics.

HLA Matching Requirements For Unrelated Donors At The SCCA/Fred Hutchinson Allied System

Acceptable levels of recipient-donor mismatch for research related treatment protocols or standard treatment plans include the following:

Allele-match for HLA-A, B, C, DRB1 and DQB1.
Single allele disparity for HLA-A, B, C, or DRB1 or DQB1
Two allele disparities for HLA-A, B, or C.
Single allele disparity for HLA-DRB1 and/or a single DQB1 antigen or allele disparity.
Single antigen plus single allele disparity for HLA-A, B, or C.

The following levels of patient-donor mismatch should be restricted to research protocols:

Two antigen disparity, either HLA-A plus C or HLA-B plus C.
Single antigen disparity for HLA-DRB1 with or without DQB1 allele or antigen disparity
Combined disparity of class I and class II loci, i.e. disparity for HLA-A, or B, or C, and any additional disparity for DRB1 or DQB1

Donor Exclusion Criteria to be considered for protocols or standard treatment plans include:

Double locus disparity. Two disparities are not allowed when they both involve the same locus, i.e., the patient is A*0101, A*0201 and the donor is A*0102 and A*0205.
Recipient and donor homozygous at mismatched locus. Patient and donor pairs homozygous at the mismatched locus are considered a two-locus mismatch, i.e., the patient is A*0101 and the donor is A*0201, and this type of mismatch is not allowed.
Recipient homozygous at mismatched locus. If the recipient is homozygous at HLA-A, B, or C and the donor is mismatched at that locus, i.e., patient is A*0101 and donor is A*0101 and A*0201, the risk of rejection is increased. Such a donor should be avoided if there is already an appreciable risk of rejection, i.e., in patients with CML/MDS/Severe Aplastic Anemia (SAA) or those receiving reduced conditioning.

Relevance of HLA matching for transplantation of unrelated hematopoietic cells:
Human Leukocyte Antigen (HLA) typing of patients and prospective hematopoietic stem cell (HSC) donors is carried out to identify and match for HLA determinants associated with successful HSC transplant outcome. While several preliminary studies (1, 2, 3) suggested the importance of allele level matching in hematopoietic cell transplantation (HCT), recent comprehensive studies confirmed that allele-level typing and matching is necessary to optimize clinical outcome in hematopoietic cell transplantation (4, 5, 6, 7).

The pervasiveness of occult HLA mismatch was shown by Petersdorf, et al in an analysis of 300 CML/CP unrelated donor-recipient pairs matched for HLA-A and B by serologic typing, and matched for the DRB1 alleles. (4) The percent of patient-donor pairs found to be matched at the allele level for all 5 loci (HLA-A, B, C, DRB1, DQB1) was only 47% (n=142). High resolution typing demonstrated previously undetected mismatches in 53% (158), indeed 26% (79) pairs were mismatched for multiple alleles. Mismatch of class I HLA was found at one locus in 55 pairs (18%) and at two or more loci in 35 pairs (12%). A single mismatch of class II HLA was detected in 24 pairs (8%), whereas 7 pairs (2%) had multiple class II mismatches, and 37 pairs (12%) had multiple mismatches involving both class I and class II. These data show the

importance of high resolution typing for defining the degree of mismatching between potential unrelated patient-donor pairs.

The degree of HLA mismatch, as well as the locus of mismatch, influence the development of alloimmune reactions and have significant implications for the outcome of HSC transplants. Studies of patient-donor pairs have shown an increased risk for graft failure with multiple mismatches that involve at least one class I allele. The incidence of graft failure was 29% in pairs where the mismatch involved more than one class I allele mismatch and 12% for mismatches involving both class I and class II alleles, compared with 2% or less for pairs with either no mismatch or mismatch confined to a single HLA-A, B, C, DRB1 and DQB1 allele. The risk of developing grades III-IV acute GVHD also has been shown to be influenced by the number and class of mismatched alleles. In studies involving primarily Caucasian patient-donor pairs, the highest risk for severe acute GVHD was observed for multiple mismatches involving both class I and class II alleles (2.0 hazard ratio and p=0.02). Pairs with a single class I mismatch did not have a significant increase in acute GVHD compared with matched recipients, but a single class II mismatch or multiple class I mismatches both appeared to confer a higher (though not significant) hazard of severe GVHD. As results of future studies further define risks of mismatches, particularly in nonCaucasian populations, we may be able to delineate more precisely “low risk” from “high risk” mismatches. Until then, the donor selection process should endeavor to identify the best matched donor within the time allowed by the clinical situation.

References:

1. Petersdorf EW, Longton GM, Anasetti C, Martin PJ, Mickelson EM, Smith AG, Hansen JA. The significance of HLA-DRB1 matching on clinical outcome after HLA-A, B, DR identical unrelated donor marrow transplantation. Blood 1995: 86:1606-1613.
2. Petersdorf EW, Longton GM, Anasetti C, Mickelson EM, Smith AG, Martin PJ, Hansen JA. Definition of HLA-DQ as a transplantation antigen. Proc. Natl. Acad. Sci. USA 1996; 93: 15358.
3. Petersdorf EW, Longton GM, Anasetti C, Mickelson EM, McKinney SK, Smith AG, Martin PJ, Hansen JA. Association of HLA-C disparity with graft failure after marrow transplantation from unrelated donors. Blood 1997: 89: 1818.
4. Petersdorf EW, Gooley TA, Anasetti C, Martin PJ, Smith AG, Mickelson EM, Woolfrey AE, Hansen JA. Optimizing outcome after unrelated marrow transplantation by comprehensive matching of HLA class I and II alleles in the donor and recipient. Blood 1998: 92: 3515.
5. Sasazuki T, Juji T, Morishima Y, Kinukawa N, Kashiwabara H, Inoko H, Yoshida T, Kimura A, Akaza T, Kamikawaji N, Kodera Y, Takaku F. Importance of HLA-class I allele matching for clinical outcome after unrelated donor hematopoietic stem cell transplantation. New Engl J Med 1998: 339: 1177.
6. Hansen JA, Yamamoto K, Petersdorf E, Sasazuki T. The role of HLA matching in hematopoietic cell transplantation. Reviews in Immunogenetics 1999: 1: 359.
7. Petersdorf EW, Hansen JA, Martin PJ, Woolfrey A, Malkki M, Gooley T, Storer B, Mickelson E, Smith A, Anasetti C. Major-histocompatibility-complex class I alleles and antigens in hematopoietic-cell transplantation. New Engl J Med 2001: 345: 1794.
8. Bodmer JG, Marsh SGE, Albert ED, Bodmer WF, Bontrop RE, Dupont B, Erlich HA, Hansen JA, Mach B, Mayr WR, Parham P, Petersdorf EW, Sasazuki T, Schreuder GMTh, Strominger JL, Svejgaard A, Terasaki PI. Nomenclature for factors of the HLA system, 1998. Tissue Antigens 1999: 53: 407.
9. List of known HLA allele sequences: **www.anthonynolan.org.uk/HIG/data.html**.
10. Information on HLA complex: **http://www.ebi.ac.uk/imgt/hla/intro.html.**

References continued:

11. Little AM and Parham P. Polymorphism and evolution of HLA class I and II genes and molecules. Reviews in Immunogenetics 1999: 1: 105.
12. Schreuder GMTh, Hurley CK, Marsh SGE, Lau M, Maiers M, Kollman C, Noreen H. The HLA Dictionary 2001: A summary of HLA-A, B, C, DRB1/3/4/5, DQB1 alleles and their association with serologically defined HLA-A, B, C, DR, and DQ antigens. Tissue Antigens 2001: 58: 109-140.
13. Smith A, McKinney S, Menninger O, Warnock S, Hansen JA. Detection of a novel "nonexpression" HLA-B*15 allele adds to data on the frequency of class I expression variants in a hematopoietic transplant population. Human Immunology 29th Annual ASHI Meeting Abstracts 2003: 64: S159.
14. Hurley CK, Baxter Lowe LA, Logan B, Karanes C, Anasetti C, Weisdorf D, Confer DL. National marrow donor program HLA-matching guidelines for unrelated marrow transplants. Biology of Blood and Marrow Transplantation 2003: 9.
15. Petersdorf, E.W., Anasetti, C., Martin, P.J., Gooley, T., Radich, J., Malkki, M., Woolfrey, A., Smith, A., Mickelson, E., Hansen, J.A. Limits of HLA mismatching in unrelated hematopoietic cell transplantation. Blood, (in press).

Approved by the Standard Practice Committee January 16, 2002 (Donor Selection Matching Requirements.doc)
HLA_matching_for_URD_selection.doc
Approved by the Medical Advisory Committee (Medical Exec. Comm.) April 9, 2002
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Revised by the Standard Practice Committee June 15, 2005
Minor wording change approved by SPC chairperson March 13, 2006

Appendix P
Adapted from COMMON TOXICITY CRITERIA (CTC)

ALLERGY/IMMUNOLOGY		
Adverse Event	**Grade 3**	**Grade 4**
Allergic reaction/ hyper-sensitivity (including drug fever)	Symptomatic bronchospasm, requiring parenteral medication(s), with or without urticaria; allergy-related edema/angioedema	Anaphylaxis
Vasculitis	Requiring steroids	Ischemic changes or requiring amputation
Allergy/Immunology – Other (specify):_____	Severe	Life-threatening or disabling
BLOOD/BONE MARROW		
Adverse Event	**Grade 3**	**Grade 4**
Hemolysis (e.g., immune hemolytic anemia, drug-related hemolysis, other)	Requiring transfusion and/or medical intervention (e.g., steroids)	Catastrophic consequences of hemolysis (e.g., renal failure, hypotension, bronchospasm, emergency splenectomy)
For BMT studies, if specified in the protocol. For pediatric BMT studies, if specified in the protocol.	>4 u pRBC in 24 hours >30mL/kg in 24 hours	Hemorrhage or hemolysis associated with life-threatening anemia; medical intervention required to improve hemoglobin Hemorrhage or hemolysis associated with life-threatening anemia; medical intervention required to improve hemoglobin
CARDIOVASCULAR - ARRHYTHMIA		
Adverse Event	**Grade 3**	**Grade 4**
Cardiovascular/Arrhythmia - Other (specify): _____ _____	Symptomatic, and requiring treatment of underlying cause	Life-threatening (e.g., arrhythmia associated with CHF, hypotension, syncope, shock)
CARDIOVASCULAR - GENERAL		
Adverse Event	**Grade 3**	**Grade 4**
Acute vascular leak syndrome	Respiratory compromise or requiring fluids	Life-threatening; requiring pressor support and/or ventilatory/support
Cardiac-ischemia/infarction	Angina without evidence of infarction	Acute myocardial infarction

Appendix P (cont'd)
Adapted from COMMON TOXICITY CRITERIA (CTC)

CARDIOVASCULAR - GENERAL (cont'd)		
Adverse Event	**Grade 3**	**Grade 4**
Cardiac left ventricular function	CHF responsive to treatment	Severe or refractory CHF or requiring intubation
Cardiac troponin I (cTnI)	Levels consistent with unstable angina as defined by the manufacturer	Levels consistent with myocardial infarction as defined by the manufacturer
Cardiac troponin T (cTnT)	≥ 0.1 - <0.2ng/mL	≥ 0.2ng/mL
Hypotension	Requiring therapy and sustained medical attention, but resolves without persisting physiologic consequences	Shock (associated with acidemia and impairing vital organ function due to tissue hypoperfusion)
Myocarditis	CHF responsive to treatment	Severe or refractory CHF
Pericardial effusion/ pericarditis	With physiologic consequences	Tamponade (drainage or pericardial window required)
Syncope (fainting) is graded in the Neurology category.	-	-
Thrombosis/embolism	Deep vein thrombosis, requiring anticoagulant therapy	Embolic event including pulmonary embolism
Vein/artery operative injury is graded as Operative injury of vein/artery in the Cardiovascular (general) category.		
Cardiovascular/General – Other (specify):_____	Severe	Life-threatening or disabling

Appendix P (cont'd)
Adapted from COMMON TOXICITY CRITERIA (CTC)

COAGULATION		
Adverse Event	**Grade 3**	**Grade 4**
DIC (disseminated intravascular coagulation) Also consider Platelets. *Note: Must have increased fibrin split products or D-dimer in order to grade as DIC.*	Laboratory findings present with no bleeding	Laboratory findings and bleeding
Thrombotic microangiopathy (e.g., thrombotic thrombocytopenic purpura/TTA or hemolytic uremic syndrome/HUS) Also consider Hemoglobin, platelets, creatinine. *Note: Must have micro-angiopathic changes on blood smear (e.g., schistocytes, helmet cells, red cell fragments).*	Laboratory findings present without clinical consequences Evidence of RBC destruction with creatinine (>3 x ULN) not requiring dialysis	Laboratory findings and clinical consequences, (e.g., CNS hemorrhage/bleeding or thrombosis/embolism or renal failure) requiring therapeutic intervention Evidence of RBC destruction with renal failure requiring dialysis and/or encephalopathy.
Coagulation - Other (specify): _____	Severe	Life-threatening or disabling
CONSTITUTIONAL SYMPTOMS		
Adverse Event	**Grade 3**	**Grade 4**
Weight gain associated with Veno-Occlusive Disease (VOD) for BMT studies, if specified in the protocol. Also consider Ascites Edema, Pleural effusion (non-malignant).	>10% or as ascites	>10% or fluid retention resulting in pulmonary failure
DERMATOLOGY/SKIN		
Adverse Event	**Grade 3**	**Grade 4**
Erythema multiforme (e.g., Stevens-Johnson syndrome, toxic epidermal necrolysis)	Severe or requiring IV fluids (e.g., generalized rash or painful stomatitis)	Life-threatening (e.g., exfoliative or ulcerating dermatitis or requiring enteral or parenteral nutritional support)

Appendix P (cont'd)

Adapted from COMMON TOXICITY CRITERIA (CTC)

DERMATOLOGY/SKIN (cont'd)		
Adverse Event	**Grade 3**	**Grade 4**
Rash/desquamation associated with graft versus host disease (GVHD) for BMT studies, if specified in the protocol.	Symptomatic generalized erythroderma or symptomatic macular, papular or vesicular eruption, with bullous formation, or desquamation covering ≥50% of body surface area.	Generalized exfoliative dermatitis or ulcerative dermatitis or bullous formation
GASTROINTESTINAL		
Ascites (none-malignant)	Symptomatic, requiring therapeutic paracentesis	Life-threatening physiologic consequences
Colitis Also consider Hemorrhage/ bleeding with grade 3 or 4 thrombocytopenia, hemorrhage/bleeding without grade 3 or 4 thrombocytopenia, melena/GI bleeding, rectal bleeding/hematochezia, hypotension.	Abdominal pain, fever, change in bowel habits with ileus or peritoneal signs, and radiographic or biopsy documentation	Perforation or requiring surgery or toxic megacolon
Diarrhea associated with graft versus host disease (GVHD) for BMT studies, if specified in the protocol. *For pediatric BMT studies, if specified in the protocol.* Also consider Hemorrhage/ bleeding with grade 3 or 4 thrombocytopenia, hemorrhage/bleeding without grade 3 or 4 thrombocytopenia, pain, dehydration, hypotension.	>1500mL of diarrhea/day *>15mL/kg of diarrhea/day*	Severe abdominal pain with or without ileus
Duodenal ulcer (requires radiographic or endoscopic documentation)	Uncontrolled by outpatient medical management; requiring hospitalization	Perforation or bleeding, requiring emergency surgery

Appendix P (cont'd)

Adapted from COMMON TOXICITY CRITERIA (CTC)

GASTROINTESTINAL (cont'd)		
Adverse Event	**Grade 3**	**Grade 4**
Gastric ulcer (requires radiographic or endoscopic documentation) Also consider Hemorrhage/bleeding with grade 3 or 4 thrombocytopenia, hemorrhage/bleeding without grade 3 or 4 thrombocytopenia.	Bleeding without perforation, uncontrolled by outpatient medical management; requiring hospitalization or surgery	Perforation or bleeding, requiring emergency surgery
Gastritis Also consider Hemorrhage/bleeding with grade 3 or 4 thrombocytopenia, hemorrhage/bleeding without grade 3 or 4 thrombocytopenia.	Uncontrolled by out-patient medical management; requiring hospitalization or surgery	Life-threatening bleeding, requiring emergency surgery
Pancreatitis Also consider Hypotension. *Note: Amylase is graded in the METABOLIC/LABORATORY category.*	Abdominal pain with pancreatic enzyme elevation	Complicated by shock (acute circulatory failure)
Mucositis *Note: Radiation-related mucositis is graded as Mucositis due to radiation.*	Painless erythema, edema, or ulcers preventing swallowing or requiring hydration or parenteral (or enteral) nutritional support	Severe ulceration requiring prophylactic intubation or resulting in documented aspiration pneumonia
Typhlitis (inflammation of the cecum) Also consider Hemorrhage/bleeding with grade 3 or 4 thrombocytopenia, hemorrhage/bleeding without grade 3 or 4 thrombocytopenia, hypotension, febrile neutropenia.	Abdominal pain, diarrhea, fever, and radiographic or biopsy documentation	Perforation, bleeding or necrosis or other life-threatening complication requiring surgical intervention (e.g., colostomy)

Appendix P (cont'd)

Adapted from COMMON TOXICITY CRITERIA (CTC)

HEMORRHAGE		
Notes: *Transfusion in this section refers to pRBC infusion.* *For any bleeding with grade 3 or 4 platelets (<50,000), always grade Hemorrhage/bleeding with grade 3 or 4 thrombocytopenia. Also consider Platelets, Transfusion: pRBCs, and Transfusion: platelets in addition to grading severity by grading the site or type of bleeding.* *If the site or type of Hemorrhage/bleeding is listed, also use the grading that incorporates the site of bleeding: NS Hemorrhage/bleeding, Hematuria, Hematemesis, Hemoptysis, Hemorrhage/bleeding with surgery, Melena/lower GI bleeding, Petechiae/purpura (Hemorrhage/bleeding into skin), Rectal bleeding/ hematochezia, Vaginal bleeding.*		
Adverse Event	**Grade 3**	**Grade 4**
Hemorrhage/bleeding with grade 3 or 4 thrombocytopenia Also consider Platelets, hemoglobin, transfusion: platelets, transfusion: pRBCs, site or type of bleeding. If the site is not listed, grade as Hemorrhage – Other (specify site):_____ *Note: This adverse event must be graded for any bleeding with grade 3 or 4 thrombocytopenia.*	Requiring transfusion	Catastrophic bleeding, requiring major non-elective intervention

Appendix P (cont'd)

Adapted from COMMON TOXICITY CRITERIA (CTC)

HEMORRHAGE (cont'd)		
Adverse Event	**Grade 3**	**Grade 4**
CNS hemorrhage/bleeding	Bleeding noted on CT or other scan with no clinical consequences	Hemorrhagic stroke or hemorrhagic vascular event (CVA) with neurologic signs and symptoms
Hemoptysis	Requiring transfusion	Catastrophic bleeding, requiring major non-elective intervention
Melena/GI bleeding	Requiring transfusion	Catastrophic bleeding, requiring major non-elective intervention
Rectal bleeding/hematochezia	Requiring transfusion	Catastrophic bleeding, requiring major non-elective intervention
Vaginal bleeding	Requiring transfusion	Catastrophic bleeding, requiring major non-elective intervention
Hemorrhage – Other (specify site): _____	Requiring transfusion	Catastrophic bleeding, requiring major non-elective intervention
HEPATIC		
Adverse Event	**Grade 3**	**Grade 4**
Bilirubin Bilirubin associated with graft versus host disease (GVHD) for BMT studies, if specified in the protocol.	>3.0 – 10.0 x ULN >6 - <15mg/100mL	>10.0 x ULN >15mg/100mL
INFECTION/FEBRILE NEUTROPENIA		
Adverse Event	**Grade 3**	**Grade 4**
Febrile neutropenia (fever of unknown origin without clinically or microbiologically documented infection).	Present	Life-threatening sepsis (e.g., septic shock)
Infection/Febrile Neutropenia – Other (specify): _____	Severe	Life-threatening or disabling

Appendix P (cont'd)

Adapted from COMMON TOXICITY CRITERIA (CTC)

NEUROLOGY		
Aphasia, receptive and/or expressive, is graded under Speech impairment in the NEUROLOGY category.		
Adverse Event	**Grade 3**	**Grade 4**
CNS cerebrovascular ischemia	Transient ischemic event or attack (TIA)	Permanent event (e.g., cerebral vascular accident)
Leukoencephalopathy associated radiological findings	Severe increase in SAS; severe ventriculomegaly; near total white matter T2 hyperintensities or diffuse low attenuation (CT); focal white matter necrosis (cystic)	Severe increase in SAS; severe ventriculomegaly; diffuse low attenuation with calcification (CT); diffuse white matter necrosis (MRI)
Seizure(s)	Seizure(s) in which consciousness is altered	Seizures of any type which are prolonged, repetitive, or difficult to control (e.g., status epilepticus, intractable epilepsy)
PULMONARY		
Adverse Event	**Grade 3**	**Grade 4**
Adult Respiratory Distress Syndrome (ARDS)	-	Present
Apnea	Present	Requiring intubation
Carbon monoxide diffusion capacity (DLCO)	>25 - <50% of pretreatment or normal value	<25% of pretreatment or normal value
FEV1	>25 - <50% of pretreatment or normal value	<25% of pretreatment or normal value
Hypoxia	Decreased O2 saturation at rest, requiring supplemental oxygen	Decreased O2 saturation, requiring pressure support (CPAP) or assisted ventilation

Appendix P (cont'd)

Adapted from COMMON TOXICITY CRITERIA (CTC)

RENAL/GENITOURINARY		
Adverse Event	**Grade 3**	**Grade 4**
Creatinine *Note: Adjust to age-appropriate levels for pediatric patients.*	>3.0- 6.0 x ULN	>6.0 x ULN
Renal failure	Requiring dialysis, but reversible	Requiring dialysis and irreversible
SECONDARY MALIGNANCY		
Adverse Event	**Grade 3**	**Grade 4**
Secondary Malignancy – Other (specify type): _____ *Excludes metastasis from initial primary.*	-	Present

Appendix Q

The Hematopoietic Cell Transplant-Comorbidity Index (HCT-CI) **9/7/10**

Assign scores appropriately if the patient has any of these comorbidities

Patient _____ *(name), UPN*_____ *Date*_____

Instructions: Circle applicable scores and provide actual value or cause of co-morbidity. Fax to FHCRC w/registration.

Comorbidities	**Definitions**	**HCT-CI scores**	**Actual Lab Values/Comments**
Arrhythmia	**Atrial fibrillation or flutter, sick sinus syndrome, and ventricular arrhythmias requiring treatment in the patient's past history**	**1**	
Cardiac	**Coronary artery disease†, congestive heart failure, myocardial infarction in patient's past history or EF of ≤50% at time of HCT**	**1**	
Inflammatory bowel disease	**Crohn's disease or ulcerative colitis requiring treatment in the patient's past history**	**1**	
Diabetes	**Requiring treatment with insulin or oral hypoglycemic, but not diet alone, at time of HCT**	**1**	
Cerebro-vascular disease	**Transient ischemic attack or cerebro-vascular accident in patient's past history**	**1**	
Psychiatric disturbance	**Depression/anxiety requiring psychiatric consult or treatment at time of HCT**	**1**	
Hepatic – mild	**Chronic hepatitis, Bilirubin >ULN- 1.5 X ULN, or AST/ALT >ULN-2.5XULN at time of HCT**	**1**	
Obesity	**Patients with a BMI of >35 for adults or with BMI-for-age percentile of ≥ 95th percentile for children at time of HCT**	**1**	
Infection	**Documented infection or fever of unknown etiology requiring anti-microbial treatment before, during and after the start of conditioning regimen**	**1**	
Rheumatologic	**SLE, RA, polymyositis, mixed CTD, polymyalgia rheumatica in patient's past history**	**2**	
Peptic ulcer	**Requiring treatment in patient's past history**	**2**	
Renal	**Serum creatinine >2 mg/dl, on dialysis, or prior renal transplantation at time of HCT**	**2**	
Moderate pulmonary	**DLco and/or FEV1 >65%-80% or Dyspnea on slight activity at time of HCT**	**2**	
Prior solid tumor	**Treated at any time point in the patient's past history, excluding non-melanoma skin cancer**	**3**	
Heart valve disease	**At time of HCT excluding mitral valve prolapse**	**3**	
Severe pulmonary	**DLco and/or FEV1 ≤65% or Dyspnea at rest or requiring oxygen at time of HCT**	**3**	
Moderate/severe hepatic	**Liver cirrhosis, Bilirubin >1.5 X ULN, or AST/ALT >2.5XULN at time of HCT**	**3**	
Please provide (KPS):	**Karnofsky Performance Score = _____%**	**Total Score =_____**	**Signature of Provider: _____**

†One or more vessel-coronary artery stenosis, requiring medical treatment, stent, or bypass graft.

EF indicates ejection fraction; ULN, upper limit of normal; SLE, systemic lupus erythmatosis; RA, rheumatoid arthritis; CTD, connective tissue disease; DLco, diffusion capacity of carbon monoxide; FEV_1, forced expiratory volume in one second; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Appendix R

WHO classification and IPSS score

WHO Classification and criteria for the myelodysplastic syndromes

Disease	Blood findings	Bone marrow findings
Refractory anemia (RA)	Anemia No or rare blasts	Erythroid dysplasia *only* < 5% blasts < 15% ringed sideroblasts
Refractory anemia with ringed sideroblasts (RARS)	Anemia No blasts	Erythroid dysplasia *only* ≥ 15% ringed sideroblasts < 5% blasts
Refractory cytopenia with multilineage dysplasia (RCMD)	Cytopenias (bicytopenia or pancytopenia) No or rare blasts No Auer rods $< 1 \times 10^9$/L monocytes	Dysplasia in ≥ 10% of cells in 2 or more myeloid cell lines < 5% blasts in marrow No Auer rods < 15% ringed sideroblasts
Refractory cytopenia with multilineage dysplasia and ringed sideroblasts (RCMD-RS)	Cytopenias (bicytopenia or pancytopenia) No or rare blasts No Auer rods $< 1 \times 10^9$/L monocytes	Dysplasia in ≥ 10% of cells in 2 or more myeloid cell lines ≥ 15% ringed sideroblasts < 5% blasts No Auer rods
Refractory anemia with excess blasts-1 (RAEB-1)	Cytopenias < 5% blasts No Auer rods $< 1 \times 10^9$/L monocytes	Unilineage or multilineage dysplasia 5% to 9% blasts No Auer rods
Refractory anemia with excess blasts-2 (RAEB-2)	Cytopenias 5% to 19% blasts Auer rods ± $< 1 \times 10^9$/L monocytes	Unilineage or multilineage dysplasia 10% to 19% blasts Auer rods ±
Myelodysplastic syndrome, unclassified (MDS-U)	Cytopenias No or rare blasts No Auer rods	Unilineage dysplasia in granulocytes or megakaryocytes < 5% blasts No Auer rods
MDS associated with isolated del(5q)	Anemia < 5% blasts Platelets normal or increased	Normal to increased megakaryocytes with hypolobated nuclei < 5% blasts No Auer rods Isolated del(5q)

Appendix R (cont'd)
WHO classification and IPSS score

IPSS score

IPSS* for MDS: Survival and AML Evolution

Prognostic Value	Score Value				
	0	0.5	1.0	1.5	2.0
BM blasts (%)	<5	5-10	—	11-20	21-30
Karyotype†	Good	Intermediate	Poor		
Cytopenias	0 or 1	2 or 3			

Scores for risk groups are as follows: Low = 0; INT-1 = 0.5-1.0; INT-2 = 1.5-2.0; and High = ≥2.5.

* International Prognostic Scoring System [21]

† Good, normal, -Y, del(5q), del(20q); Poor, complex (≥3 abnormalities) or chromosome 7 anomalies; Intermediate, other abnormalities.

Appendix S

Weight / Adjusted Body Weight for Drug Dosing

weight_for_drug_dosing.pdf

Appendix T

Radiotherapy Treatment Guidelines per Standard Practice


TBI_Adult_Non_Myeloablative.pdf


TBI_Pediatric_NON_Myeloablative.pdf

Appendix U

COORDINATING CENTER FUNCTIONS
Outside Center – PI Communication in Hematologic Malignancies

I. Study Management, data analysis, and Data and Safety Monitoring

a. Study Management:
   i. Each local PI is responsible for selection, training and oversight of local study coordinators
   ii. The Coordinating Center registers subjects on the study and assigns study IDs
   iii. One copy of the research data is retained by the site. Another data set (identified only by study IDs) is transmitted to the Coordinating Center to create the master data file. All data are kept in locked areas and password protected databases accessible only to study staff
   iv. The quality of data is monitored in an ongoing fashion with the study team and corrective action plans instituted as necessary

b. Data Analysis:
   i. Study staff review data for completeness as it is submitted by the sites
   ii. The study statistician is responsible for data cleaning and the conduct of analyses as outlined in the protocol and grant

c. Data Safety and Monitoring:
   i. The trial coordinators at collaborating centers or the local PIs will report SAEs (as defined by the protocol) to the Coordinating Center and an official report of an SAE is faxed to the Coordinating Center within ten days
   ii. The SAE report is reviewed by the Overall PI. If the SAE meets the FHCRC criteria for expedited reporting then an official signed report is submitted to the IRB
   iii. An independent DSMB will meet at six-month intervals and all outcome data is reviewed including all adverse events and SAEs reported to the Coordinating Center along with those officially reported to the IRB
   iv. A report from the DSMB is submitted to the IRB as well as the trial coordinators/local PIs participating in the protocol

II. Protocol and informed consent document management

a. A master protocol is maintained by the Coordinating Center and distributed to the sites for customization and local IRB review
b. All protocol and consent modifications initiated by the Coordinating Center are sent to the Collaborating Sites following approval by the Coordinating Center IRB, for review and approval by the local IRB
c. Changes required by local IRBs are reviewed by the Coordinating Center and approved prior to implementation at local sites

III. Assurance of local IRB OHRP-approved assurance

a. Each site provides their OHRP assurance number and evidence of IRB certification
b. Study staff monitor maintenance of institutional assurance and IRB certification

IV. Assurance of local IRB approvals

a. The Coordinating Center maintains copies of the most current collaborating site Consent Forms and IRB approval documentation
b. No site may enroll subjects until the Coordinating Center has received confirmation of local IRB approval
c. Each site is responsible for preparation and submission of their continuing reviews. Any changes to the protocol or consent form will be communicated to the Coordinating Center
d. Sites are required to have active IRB approvals to participate in any study related activities

V. Any substantive modification by the Collaborating Institution related to risks or alternative procedures is appropriately justified

a. The Coordinating Center reviews any modifications to consent forms to ensure that site consents do not delete or change the basic or additional elements or alternatives required in the sample consent form

VI. Informed consent is obtained from each subject in compliance with HHS regulations

a. Subjects must provide written informed consent prior to study participation
b. The Coordinating Center verifies eligibility and signed consent prior to assigning a study ID number

APPENDIX V
Standard Donor Consent

standard donor consent.pdf